UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MEMORANDUM

DATE: 27 July 2006

SUBJECT: Furfural. Revised Human Health Risk Assessment for Greenhouse Soil Fumigation with a New Active Ingredient.

PC Code:	043301	DP Barcode:	D328940
Trade Name:	MULTIGAURD™ PROTECT	EPA Reg #:	75753-XXX
Class:	Fumugant - Nematicide/Fungicide		

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The Health Effects Division (HED) of the Office of Pesticide Programs (OPP) is charged with estimating the risk to human health from exposure to pesticides. The Registration Division (RD) of OPP has requested that HED conduct revised occupational, residential, and aggregate exposure assessments, as needed, to estimate the risk to human health that will result from the proposed use of furfural in greenhouses as a soil fumigant for ornamentals and other non-food commodities. This document contains revisions to the previous memo (D316219, K. O'Rourke, 10/13/05) that are based new information provided by the registrant, including: changes to the proposed label indicating a reduction in the pre-plant application rate, and a prohibition of the use of sterile soil/growing media; new dislodgeable foliar residue data; and industry practices regarding air exchange rates used in greenhouses.

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INTRODUCTION

A summary of the findings and a revised assessment of human health risk resulting from the proposed use of furfural is provided in this document. The HED team members contributing to this risk assessment include: Charles Smith and Jack Arthur (occupational/residential assessment), Stanley Gross and Stephen Dapson (toxicology assessment), and Kelly O'Rourke (overall risk assessment).

Recommendation for Registration

Provided the data specified in Section 8.0 of this risk assessment are submitted, and the required label revisions are made, this human health risk assessment does not preclude a conditional registration for the proposed use of furfural in greenhouses, based on the need for: a greenhouse volatility study, a dislodgeable foliar residue study, an acceptable 28-day dermal toxicity study, and a guideline 90- or 28-day inhalation study.

1.0 EXECUTIVE SUMMARY

The Health Effects Division (HED) has conducted a revised screening level human health risk assessment for the new active ingredient furfural for the purpose of making a registration eligibility decision to establish use in greenhouses as a soil fumigant for ornamentals.

Furfural is a new active ingredient proposed as a fumigant to control root infesting plant parasitic nematodes, and fungal plant diseases in greenhouse soil used for growing ornamentals and other non-food commodities. Furfural is a by-product of sugar cane processing.

Proposed Uses

In this action, the end-use product containing 90% furfural in a liquid formulation (MULTIGUARD™ PROTECT) is proposed for use in growing media and/or soils in greenhouses for cut flowers, cut greens, transplants, propagative materials, ornamentals and other non-food/non-feed commodities. The proposed furfural end-use product is packaged as a 90% furfural liquid formulation. This use was previously assessed in D316219 (K. O'Rourke, 10/13/05). This revised assessment reflects new information provided by the registrant, including:

- revision of the proposed label indicating a reduction in the pre-plant application rate from 540 lb ai/A to 45 lb ai/A (i.e., equal to the post-plant application rate);
- revision of the proposed label indicating that sterile soil/growing media may not be used;
- new dislodgeable foliar residue data from a non-guideline study on poinsettias (MRID# 46809701); and
- information provided during numerous meetings with the registrant, and in comment submissions regarding the previous risk assessment, particularly industry practices regarding air exchange rates used in greenhouses.

The following additional documents were considered in the development of this assessment:

- *Exposure and Risk Assessment for Indoor Non-Residential Use of Multiguard Protect on Non-Food Crops: (Furfural)*, G. Whitmyre (MRID# 46331507);
- *Mixer/Loader, Applicator, and Reentry Worker Risk Assesment Associated with Drench Application of Multigard Protect on Non-Food Crops in Greenhouses, Shade Houses and Nurseries*, G. Whitmyre (MRID# 46426510);
- *Occupational Exposure Assessment for Application of Multiguard Protect to Non-Food Crops in Greenhouses*, J. Johnston (MRID# 46009311);
- Slide presentations from meetings that took place on December 13, 2005, and February 14 and 16, 2006; and
- Comment submissions:
    - *Response to HED/EFED Risk Assessments Attached to EPA Letter (M. Waller) Dated November 4, 2005* (MRID# 46752301); and
    - *EPA File Symbol 75753-E (Furfural Technical); EPA File Symbol 75753-R (Multiguard™ Protect); Request for Conditional Registrations*, April 12, 2006.

Hazard Characterization

The acute toxicity profile for furfural ranges from highly toxic to relatively non-toxic (from Toxicity Category of I to IV). Technical furfural has a pungent odor smelling like almonds. It is irritating to skin, mucous membranes and the respiratory system. Single- and repeated dose animal toxicity studies in the open literature, using various routes and animal species, give evidence of adverse effects involving most physiological systems including respiratory system, liver and kidney, blood and bone marrow as well as adverse effects to the nervous system.

Studies in humans and animals show that furfural is readily absorbed and is excreted in the urine. The American Conference of Governmental Industrial Hygienists (ACGIH) occupational standard for furfural is Threshold Limit Value (TLV) is 2 ppm with a "Skin" notation for concerns for vapor irritation of skin and mucous membranes. The Agency has not classified the carcinogenic potential of furfural at this time, however, the National Toxicity Program (NTP) carcinogenicity study in rats and mice, submitted by the registrant, does not indicate a potential for carcinogenicity.

Dose Response Assessment

The Health Effects Division's (HED) Registration Action Branch III (RAB3) Toxicology Team, in conjunction with members of HED's Fumigant Risk Assessment Team, evaluated the hazard data and selected endpoints for dermal and inhalation exposure assessment of furfural. The dermal endpoint was based on range-finding and primary developmental toxicity studies in the rat (NOAEL = 10 mg/kg/day), in which clinical signs (bilateral exophthalmia, tremors, and head held low) were observed at the LOAEL of 50 mg/kg/day. Because this is an oral NOAEL, the estimated dermal exposures should be adjusted for dermal absorption. However, no acceptable data are available to estimate dermal absorption, therefore the standard100 percent dermal absorption rate was used in the assessment. The level of concern (LOC) for short- and intermediate-term dermal exposure to furfural is 100, based on a 10X interspecies factor and a 10X intraspecies factor. Long-term exposure is not expected for the proposed use pattern.

For inhalation exposure, human equivalent concentrations (HECs) were derived from the 28-day inhalation study and used to calculate margins of exposure using EPA's reference concentration (RfC) methodology (1994) for estimating inhalation exposures. EPA's RfC method provides algorithms which calculate HECs for different regions of the respiratory system. The HECs differ between non-occupational (0.58 mg/m^3 for acute exposures) and occupational scenarios (1.73 mg/m^3 for acute, short-, and intermediate-term exposures) since non-occupational HECs are based on 24-hour exposures occurring 7 days per week, while the occupational HECs are based on 8-hour exposures occurring 5 days per week. Because EPA's RfC methodology incorporates some pharmacokinetic differences between rats and humans, the interspecies extrapolation factor is reduced to 3x. In addition, EPA typically uses a 10x factor to account for intraspecies variability. For furfural, HED also used a 10x uncertainty factor for LOAEL to NOAEL extrapolation. Therefore, a MOE of 300 defines the LOC for short- and intermediate-term exposure to furfural.

The proposed use for the new active ingredient furfural is non-food (i.e., greenhouse ornamentals); therefore, an FQPA assessment of this chemical has not been conducted.

Residential/Bystander Exposure Estimates

Residential uses are not proposed for furfural, however, residential/bystander exposure is possible from drift of furfural vapors associated with the proposed greenhouse use. In order to conduct a reliable bystander exposure assessment, field volatility data are necessary. The Registrant did not submit this type of study in support of their registration request; therefore, a screening level assessment was conducted. The only available data are from the Registrant's laboratory soil volatility study, which raise many uncertainties when used for the purpose of assessing bystander exposure.

These laboratory data were used as inputs to EPA's Industrial Source Complex: Short-Term Model (ISCST3) to estimate furfural concentrations outside the greenhouse after a furfural application. As mentioned previously, the inhalation LOC for bystanders is an MOE of 300 or greater, below which indicate risks of concern. The modeling results indicate that, **for bystanders, a distance of 30 meters downwind may be necessary to achieve an MOE of 300 for small greenhouses, and a distance of 100 meters may be required for large greenhouses**. Please note that there is low confidence in these estimates because they are based on data that, in addition to other significant limitations, were not generated under field conditions.

Dietary Exposure Estimates

Furfural is a new active ingredient; therefore, there are no existing tolerances for this chemical. The proposed use is for greenhouse ornamentals. Because this is a non-food use, the "food only" portion of the dietary assessment is not applicable.

The proposed use pattern for furfural is considered to have minimal potential for causing drinking water contamination (e.g., the application requires 5 gallons of 90% furfural formulation, mixed with 45 gallons of water, to be applied per acre, and then to be watered-in with at least 5,445 gallons per acre). A dietary exposure assessment for drinking water was not conducted.

Aggregate Exposure Scenarios and Risk Conclusions

As mentioned previously, furfural is a new active ingredient for which there are no existing tolerances, and the proposed use is for greenhouse ornamentals. For non-food uses, where there are no food residue data or tolerances, an aggregate risk assessment is not required under the FQPA, and therefore, was not conducted.

Occupational Exposure Estimates

No chemical-specific exposure data were submitted in support of this Section 3 registration for furfural. In accordance with HED policy, dermal occupational handler exposures were estimated using the Pesticide Handlers Exposure Database (PHED) Surrogate Exposure Guide (revised August, 1998). For some of the occupational handler scenarios that reflect baseline clothing, dermal occupational handler risks are of concern (i.e., the MOEs do not reach 100). However, **when gloves are added, all handler scenarios have MOEs of 100 or greater, and therefore, are not of concern.**

No chemical-specific data were available to assess potential inhalation exposures to handlers from the proposed uses. Inhalation handler risks for furfural were not assessed using PHED because furfural is much more volatile (2 mm Hg at 20 °C) than the pesticides that are incorporated into PHED. As a result, inhalation risks would be underestimated if PHED data were used to assess inhalation handler exposures. **The inhalation postapplication exposures and risks can be considered a surrogate to represent inhalation handler exposures and risks.**

The registrant recently submitted a non-guideline dislodgeable foliar residue (DFR) study conducted on poinsettias (MRID# 46809701) for use in assessing potential dermal exposures to postapplication workers from the proposed furfural uses. These data have been reviewed and are considered to be of poor quality, however, they have been used in this assessment as surrogate data until the required guideline DFR study is complete. The postapplication exposure assessment indicates that dermal occupational risks are of concern (i.e., the MOEs are less than 100) on day 0, and up to 9 days following application, depending on the scenario. **Interim restricted entry intervals (REIs) are estimated to be 12 hours for containerized ornamentals, and 9 days for cut flowers.**

No chemical-specific data were available to assess potential inhalation exposures to postapplication workers from the proposed furfural uses. In this instance, HED utilized EPA's Multi-Chamber Concentration and Exposure Model (MCCEM) to estimate furfural concentrations inside the greenhouse after a furfural application. For all greenhouse postapplication exposure scenarios, inhalation postapplication occupational risks are of concern (i.e., the MOEs are less than 300) on day 0 using worst-case air exchange rates. **Postapplication inhalation MOEs do not reach 300 until the air exchange rates are increased to 65 per hour (based on 8-hour average) or 90 per hour (based on 1-hour average).**

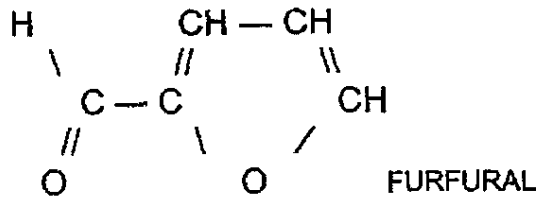
Recommendation for Registration

Provided the data specified in Section 8.0 of this risk assessment are submitted, and the required label revisions are made, this human health risk assessment does not preclude a conditional registration for the proposed use of furfural in greenhouses, based on the need for: a greenhouse volatility study, a dislodgeable foliar residue study, an acceptable 28-day dermal toxicity study, and a guideline 90- or 28-day inhalation study.

2.0 PHYSICAL/CHEMICAL PROPERTIES CHARACTERIZATION

Furfural is a by-product of sugar cane processing. The nomenclature and chemical structure of furfural are shown below:

Common name:	Furfural Technical
IUPAC name:	2-Furaldehyde or furfural
CAS name:	2-Furancarboxaldehyde
CAS #:	98-01-01



2.1 Physical and Chemical Properties

The product chemistry data for furfural were reviewed by the Registration Division (D295324, 2/26/04, L. Kutney). Furfural is an oily liquid with an almond-like odor characteristic of aldehydes. It is yellow in color, turning reddish-brown to black on exposure to air.

Molecular Weight:	96.1 g/mol
Boiling point:	161.7°C
Density:	1.16g/ml at 20°C
Water solubility (20°C):	7.81 g/100 ml
Solvent solubility (mg/L at 20°C):	alcohol (infinite) ether (infinite) miscible in octanol, acetone, xylene, ethyl acetate, methylene chloride and methanol
Vapor pressure:	2.6 mm Hg (at 20°C)
Dissociation constant (pK_a):	Does not demonstrate a dissociation constant between pKa2 and pKa10.
Octanol/water partition coefficient Log(K_{OW}):	0.35 at 20°C
UV/Visible absorption:	14591.3 cm^2/mole (pH 7) 15324.2 cm^2/mole (pH 1.94) 14584.8 cm^2/mole (pH 10.12)

3.0 Hazard Characterization/Assessment for Furfural

3.1 Hazard Characterization

The studies submitted in support of the registration are limited in scope with most of the hazard information obtained from the open literature. The available open literature database is old, but extensive and includes information on the use, occurrence and hazards of furfural. Some of the information presented in this section is based on the study summaries (rather than full study submissions), and these summaries would need additional support (raw data for analysis) to be used for regulatory purposes.

Technical furfural is a by-product of sugar cane production. The FDA (Food and Drug Administration) considers levels of furfural in natural products and the use of furfural as a flavoring agent as GRAS (Generally Recognized As Safe) and therefore assumes those levels of exposure to furfural and furfural-like ingredients as having no adverse effects when used under designated GRAS conditions.

The acute toxicity profile for furfural ranges from highly toxic to relatively non-toxic (from Toxicity Category of I to IV). Technical furfural has a pungent odor smelling like almonds. It is irritating to skin, mucous membranes and the respiratory system. Single- and repeated dose animal toxicity studies in the open literature, using various routes and animal species, give evidence of adverse effects involving most physiological systems including respiratory system, liver and kidney, blood and bone marrow as well as adverse effects to the nervous system.

Studies in humans and animals show that furfural is readily absorbed and is excreted in the urine. The American Conference of Governmental Industrial Hygienists (ACGIH) occupational standard for furfural is Threshold Limit Value (TLV) is 2 ppm with a "Skin" notation for concerns for vapor irritation of skin and mucous membranes. The Agency has not classified the carcinogenic potential of furfural at this time, however, the National Toxicity Program (NTP) carcinogenicity study in rats and mice, submitted by the registrant, does not indicate a potential for carcinogenicity.

Because the Registrant has submitted a request for a non-food greenhouse registration, a limited toxicology dataset has been submitted for review by the Agency. It includes acute toxicity studies and studies published in the open literature including a subchronic oral toxicity study in rats and mice, a chronic oral toxicity study in rats and mice, two oral developmental studies, one in rats and the other in rabbits, a 28-day dermal study in rats, 28-day inhalation study in rats, as well as a number of review articles from regulatory (primarily European) agencies. Based on the anticipated use pattern, the dermal and inhalation routes appear to be the major routes of exposure. Consequently, the current data base review focuses on these routes to assess potential hazards for worker and bystander exposures.

The Registrant has submitted the studies listed in Tables 1 and 2, which include a number of recent studies and summaries published in the open literature. These include the usual acute studies for technical furfural and the end-use product, Multiguard™ Protect containing 90% furfural. The Registrant has also submitted subchronic oral, dermal and inhalation studies as well as chronic, carcinogenicity, developmental and mutagenicity studies as shown in Table 2. The Studies missing in Table 2 have been referenced to the open literature as discussed in the following sections.

Table 1. Acute Toxicity Profile - Furfural

Guideline No.	Study Type	MRID(s)	Results	Toxicity Category
870.1100	Acute oral in rats (Rana, 2002)	46011009	LD_{50} = >102 mg/kg	II
870.1200	Acute dermal in rats (Joseph, 2003)	46011010	LD_{50} = 192 mg/kg	I
870.1300	Acute inhalation in rats. (Merkel, 2003)	46106302	LC_{50} = 0.54-1.63 mg/L	III
870.2400	Acute eye irritation in rabbits. (Joseph, 2003)	46011012	Severe. Irritant.	II
870.2500	Acute dermal irritation in rabbits. (Joseph, 2003)	46011013	Slight. Irritant.	IV
870.2600	Skin sensitization in Guinea pigs. (Joseph, 2003)	46011014	Non sensitizer.	Neg.

Table 2. Subchronic, Chronic and Other Toxicity Profile for Furfural

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Results
870.3100 90-Day oral toxicity rats	46011015, 1990 Acceptable/**Non-guideline** 0, 11, 22, 45, 90, 180 mg/kg/day	NTP 1990 Study **(publication).** NOAEL = 45 mg/kg/day LOAEL = 90 mg/kg/day based on liver pathology – cytoplasmic vacuolization of hepatocytes.
870.3100 90-Day oral toxicity mice.	46011015, 1990 Acceptable/ **Non-guideline** 0, 75, 150, 300, 600, 1200 mg/kg/day	NTP 1990 Study **(publication)**. NOAEL < 75 mg/kg/day LOAEL <= 75 mg/kg/day based on relative liver weights.
870.3100 90-Day oral toxicity rats.	46011015, 2001 Summary/**Non-guideline (WHO published review article)** 0, 30, 60, 90, 180 mg/kg/day (microencapsulated)	WHO **(published review article),** Food Additive Series 46 (2001) NOAEL = 60 mg/kg/day LOAEL = 90 mg/kg/day based on liver effects.

Table 2. Subchronic, Chronic and Other Toxicity Profile for Furfural		
Guideline No./ Study Type	**MRID No. (year)/ Classification /Doses**	**Results**
870.3150 90-Day oral toxicity dog		**No study submitted.**
870.3200 28-Day dermal toxicity in rats.	46465501 Unacceptable/**Guideline** 0, 25, 50 and 100 mg/kg	NOAEL =>100 mg/kg/day (HDT). LOAEL => 100 mg/kg/day. Transient effects included drowsiness, dyspnea, clonic convulsion, hyperactivity, tremor, vocalization, generalized effects from exposure to furfural
870.3465 28-Day inhalation toxicity in rats	46426504, -05 Acceptable/Guideline 0, 20, 40, 80, 160, 320, 640, 1280 mg/cu.m.	NTO (Netherlands) 2001 **(study publication)**. LOAEL = < 20 mg/cu.m. (LDT) causing nasal epithelium pathology. NOAEL < 20 mg/cu.m.
870.3700a Prenatal developmental in rats	46147601,1997 primary study: 0, 50, 100, 150 mg/kg/day Acceptable/Guideline w/rangefinder 46629401, 1996 rangefinder: 0, 10, 50, 100,150, 250, 500, 1000 mg/kg/day	**Maternal** NOAEL = 10 mg/kg/day (from rangefinder) LOAEL = 50 mg/kg/day (from primary) based on clinical signs. **Developmental** NOAEL => 150 mg/kg/day LOAEL > 150 mg/kg/day , no treatment related effects noted in the primary study, no relevant observations in the rangefinding study.
870.3700b Prenatal developmental in rabbits	46207303, 2004 0, 25, 75, 225 mg/kg/day 46207302, 2003 (rangefinder) 0, 25, 50, 100, 150, 300 mg/kg/day	**Maternal** NOAEL = 225 mg/kg/day LOAEL = 300 mg/kg/day based on decreased bw, bwg - primary study combined with rangefinder study data **Developmental** NOAEL = 225 mg/kg/day LOAEL = 300 mg/kg/day based on decreased fetal bw - primary study combined with rangefinder study data
870.3800 Reproduction and fertility effects		**No study available.**
870.4100a Chronic toxicity Rats	46011016, 1990 Acceptable/**Non-guideline.** 0, 30, or 60 mg/kg/day	NTP 1990 Study **(publication).** NOAEL = 30 mg/kg/day LOAEL = 60 mg/kg/day based on liver effects.
870.4100a Chronic toxicity mice	46011016, 1990 Acceptable/**Non-guideline.** 0, 50, 100, or 175 mg/kg/day	NTP 1990 Study **(publication)** NOAEL => 175 mg/kg/day LOAEL > 175 mg/kg/day
870.4100b Chronic toxicity dog		**No study submitted.**

Table 2. Subchronic, Chronic and Other Toxicity Profile for Furfural		
Guideline No./ Study Type	**MRID No. (year)/ Classification /Doses**	**Results**
870.4200 Carcinogenicity rat.	46011016, 1990 Acceptable/**Non-guideline** 0, 30, 60 mg/kg/day	NTP 1990 Study **(publication).** NOAEL = 30 mg/kg/day LOAEL = 60 mg/kg/day based on liver effects. **no evidence of carcinogenicity at dose levels tested**
870.4300 Carcinogenicity mouse.	46011016, 1990 Acceptable/**Non-guideline** 0, 50, 100, 175 mg/kg/day	NTP 1990 Study **(publication)** NOAEL => 175 mg/kg/day LOAEL > 175 mg/kg/day **no evidence of carcinogenicity at dose levels tested**
Gene Mutation 870.5100	46011017; 1999 Acceptable/Guideline	Negative for bacterial reverse mutation assay
Gene Mutation 870.5100	46011018; 2003 Acceptable/Non-guideline	Negative for *in vivo* gene mutation bacterial gene incorporation into genome of transgenic mice
CA/SCE 870.5375, 870.5385, 870.5900, 870.5915	46011019; 2003 (compilation of 7 reports)	Negative and Acceptable/Guideline: Reverse Gene Mutation, In vitro mammalian gene mutation and chromosomal aberrations , In vivo Chromosomal Aberrations, SCE, Gene Mutation – Drosophilia Unacceptable/Guideline – Expert Panel Report, SCE in human Lymphocytes, in vitro cytogenetic assays
UDS 870.5500, 870..5560	46011020; 2003 (compilation of 9 reports)	Negative for DNA damage/repair, rec-assay, UDS in rat hepatocytes – Acceptable/Guideline DNA damage, summary reports - Unacceptable
870.7485 Metabolism and pharmacokinetics		**No study submitted.**
870.7600 Dermal penetration		**No study submitted.**

Non-guideline = studies either from the open literature, studies not meeting guideline requirements, but contain useful information or range-finding studies

3.2 FQPA Hazard Considerations

Furfural is a new active ingredient proposed for use on greenhouse ornamentals. This use is nonfood; therefore, an FQPA assessment of this chemical has not been conducted.

3.2.1 Adequacy of the Toxicity Data Base

At this time, the Agency is conducting a quantitative human health risk assessment for exposure *via* the inhalation and dermal routes only. For the purpose of conducting these risk assessments, the current furfural database provides limited information to assess risks to the human population following furfural exposure. The data set consists primarily of the acute toxicity data package required for all new active ingredients, studies reported in the open literature, as well as review articles from several regulatory bodies that cite studies not readily available to Agency reviewers.

3.2.2 Evidence of Neurotoxicity

The developmental toxicity study in rats exhibited clinical signs one hour post dosing and during daily examinations, including tremors and head held low, hypoactivity, vocalization, labored respiration, rales and gasping, rapid respiration, prostrate animals, lethargic, limited use of hindlimbs and unkempt appearance.

3.2.3 Developmental Toxicity Studies

Developmental Toxicity in the rat:

In a developmental toxicity study (MRID 46147601), Furfural technical (99.4-100% a.i., Lot # 1218) was administered daily via oral gavage to 25 presumed pregnant Sprague-Dawley (Crl:CD®(SD)BR) rats/group at a dose volume of 5 mL/kg (in water) at dose levels of 0, 50, 100, or 150 mg/kg/day from gestation day (GD) 6 through 15. In the 150 mg/kg/day group, dosing was terminated on April 15, corresponding to GD 10-14, due to substantial maternal toxicity. However, the rats in this group remained on study to assess reversibility. All surviving dams were killed on GD 20; their fetuses were removed by cesarean section and examined.

Between GD 6 and 15, 3/25 dams in the 100 mg/kg/day group died, and 16/25 dams in the 150 mg/kg/day group died. Among the decedents, foamy contents in the trachea and firm lungs were noted in 1/3 dams at 100 mg/kg/day, and the following findings were noted at 150 mg/kg/day: (i) foamy contents in the trachea and firm lungs in 2/16 dams; (ii) mottled or dark red lungs in 3/16 dams; (iii) dilated renal pelvis in 2/16 dams; (iv) dark red contents in the jejunum in 1/16 dams; and (v) autolyzed intestine and complete litter resorption in 1/16 dams.

At one hour post-dosing, the following clinical signs of toxicity were observed: (i) bilateral exophthalmia, tremors, and head held low at 50 mg/kg/day and above; (ii) hypoactive, vocalization, labored respiration, rales, gasping, and rapid respiration at 100 mg/kg/day and above; and (iii) prostrate, lethargic, limited use of hindlimbs, and dried red material around mouth and right eye at 150 mg/kg/day.

During the daily clinical examinations, bilateral exophthalmia was observed at 50 mg/kg/day and above. Additionally at 150 mg/kg/day, the following clinical signs of toxicity were noted: (i) hypoactive; (ii) prostrate; (iii) tremors; (iv) head held low; (v) labored/rapid respiration; (vi) rales; (vii) decreased defecation; (viii) unkempt appearance; and (ix) numerous findings on the coat and around the eyes, nose, and mouth, including matting (clear, yellow, brown, red, wet, or dry) on the forelimbs and ventral thoracic, abdominal, and/or urogenital areas.

At 150 mg/kg/day, body weight gains and absolute and relative (to body weight) food consumption were decreased during GD 6-12, resulting in decreased body weight gains for the overall (GD 6-16) treatment interval. Body weight gains and food consumption in this group were comparable to controls during GD 12-16, corresponding to when the surviving animals in this group were taken off dose (GD 10-14).

The Maternal Toxicity NOAEL is less than 50 mg/kg/day (LDT in primary study) and the Maternal Toxicity LOAEL is equal to or less than 50 mg/kg/day based on clinical signs of toxicity (bilateral exophthalmia, tremors, and head held low).

There were no effects of treatment on the mean numbers of corpora lutea, implantations, or live fetuses per dam. Similarly, in animals surviving to scheduled sacrifice, there were no abortions, premature deliveries, dead fetuses, or complete litter resorptions, and there were no effects of treatment on the number of resorptions (early or late) or on fetal weights, sex ratio, or post-implantation loss. There were no treatment-related external, visceral, or skeletal malformations or variations.

The Developmental Toxicity NOAEL is equal to or greater than 150 mg/kg/day (HDT) and the Developmental Toxicity LOAEL is greater than 150 mg/kg/day.

A Maternal Toxicity NOAEL in the primary study was not established; therefore, the doses selected for this primary study were apparently too high. It was stated that the dose levels were selected based upon the results of a preliminary range-finding study (MRID# 46629401), this study used dose levels ranging from 10 to 1000 mg/kg/day. The comparable doses between the 2 studies were the 50, 100 and 150 mg/kg/day, based on the effects noted in the range-finding study at 150 mg/kg/day which included clinical signs and transient body weight decrease, a supportable Maternal Toxicity NOAEL of 10 mg/kg/day can be established, with a Maternal Toxicity LOAEL of 50 mg/kg/day.

Developmental Toxicity study in rabbits:

In a developmental toxicity study (MRID 46207303), Furfural technical (99.67% a.i., Lot # 0305-1373A) was administered daily via oral gavage to 25 artificially inseminated New Zealand White rabbits/group at dose levels of 0, 25, 75, or 225 mg/kg/day at a dose volume of 5 mL/kg from gestation day (GD) 0 through 28. All surviving does were killed on GD 29; their fetuses were removed by cesarean section and examined.

There were no effects of treatment on survival, body weights, body weight gains, net body weight gain (adjusted for gravid uterine weight), gravid uterine weight, absolute or relative (to body weight) food consumption, or gross pathology.

The only apparent effect of treatment was the observation of unkempt appearance in 1/24 rabbits at 75 mg/kg/day for 8 days and in 6/25 rabbits at 225 mg/kg/day for an average of 4.3 days per rabbit. Since this clinical sign was not corroborated by any other findings, it is not considered toxicologically significant.

The Maternal Toxicity NOAEL is equal to or greater than 225 mg/kg/day (HDT in primary study) and the Maternal Toxicity LOAEL is greater than 255 mg/kg/day.

There were no dead fetuses or premature deliveries. Similarly, there were no effects of treatment on the pregnancy rate, sex ratio, pre-implantation loss, post-implantation loss, or on the numbers of abortions, corpora lutea, implantations, litters, live fetuses, or resorptions (early, late, or complete litter). There were no effects of treatment on fetal body weights or on ossification of the skeleton, indicating that fetal growth and development were unaffected by treatment. There were no treatment-related external, visceral, or skeletal malformations or variations.

The Developmental Toxicity NOAEL is equal to or greater than 225 mg/kg/day (HDT) and the Developmental Toxicity LOAEL is greater than 225 mg/kg/day.

NOTE: Although neither a Maternal nor a Developmental Toxicity LOAEL was observed, Maternal Toxicity was observed at 300 mg/kg/day in the range-finding study. Thus, the dose selection rationale for the definitive study was appropriate. The Maternal Toxicity NOAEL is 225 mg/kg/day and the Maternal Toxicity LOAEL is 300 mg/kg/day.

3.2.4 Reproductive Toxicity Study

No reproduction study was provided, not required for non-food use application.

3.2.5 Additional Information from Literature Sources

There are numerous animal toxicity studies in the open literature, using various species and routes of exposure, which present evidence of adverse effects involving most physiological systems including the respiratory system, liver and kidney, blood and bone marrow as well as adverse effects to the nervous system. Mutagenicity studies are inconsistent (some positive and some negative for mutagenicity). The carcinogenicity profile for furfural is also inconsistent; while some data indicate a positive carcinogenic response other data are negative. Studies in humans and animals show that furfural is readily absorbed and is excreted in the urine. At elevated exposure levels, furfural vapors produce irritation of the eyes, skin and respiratory tract. The ACGIH occupational standard is TLV = 2 ppm with a "Skin" notation for concerns for vapor irritation of skin and mucous membranes.

3.2.6 Pre-and/or Postnatal Toxicity

3.2.6.1 Determination of Susceptibility

There is no evidence of susceptibility in the submitted developmental toxicity studies in either the rat or rabbit. However, postnatal susceptibility cannot be evaluated. A-2 generation reproduction study was not submitted since it is not required for a non-food.

3.3 Recommendation for a Developmental Neurotoxicity Study

3.3.1 Evidence that supports requiring a Developmental Neurotoxicity study

The developmental toxicity study in rats exhibited clinical signs one hour post dosing and during daily examinations, including tremors and head held low, hypoactivity, vocalization, labored respiration, rales and gasping, rapid respiration, prostrate animals, lethargic, limited use of hindlimbs and unkempt appearance.

There are no other available repeated dose studies with clinical and pathological evaluations.

3.3.2 Evidence that supports not requiring for a Developmental Neurotoxicity study

The open literature does not indicate any specific neuropathology, rather just neurotoxicity generalized signs as mentioned in Section 3.3.1 above.

3.3.2.1 Rationale for the UF_{DB} (when a DNT is recommended)

Given the limited data set available for review at this time, the Agency is placing the requirement for a DNT on reserve pending submission of additional data that may more clearly characterize the toxicity profile for Furfural.

3.4 Hazard Identification and Toxicity Endpoint Selection

Based on the proposed use patterns (greenhouse), the primary exposure pathways for Furfural are the inhalation and dermal routes. Since Furfural is considered a non-food use active ingredient and there are no residential uses, oral risk assessments (dietary and incidental oral) have not been conducted at this time. However, should use patterns change in the future to include food uses and/or residential uses, the Agency may conduct these risk assessments.

3.4.1 Dermal Absorption

No dermal absorption study was provided. The available data do not support a deviation from the default assumption of 100% dermal absorption (or a 100% inhalation absorption factor). Open literature studies in humans and animals indicate that furfural is readily absorbed through the intact skin. There is a submitted subchronic oral toxicity study that is non-guideline, with only a limited number of parameters measured (a range-finder study for an NTP carcinogenesis study and a submitted subchronic dermal study that was judged inadequate with no endpoint determined. therefore, there was no way to calculate an oral to dermal factor).

3.4.2 Dermal Exposure (Short, Intermediate and Long Term)

For all exposure periods:

Study Selected: Developmental Toxicity in the rat

MRID. No.: 46147601 and 46629401

Executive Summary: see Section 3.2.3, Developmental Toxicity Studies

Dose and Endpoint used for risk assessment: LOAEL is 50 mg/kg/day based on clinical signs of toxicity (bilateral exophthalmia, tremors, and head held low). A Maternal Toxicity NOAEL in the primary study was not established; therefore, the doses selected for this primary study were apparently too high. It was stated that the dose levels were selected based upon the results of a preliminary range-finding study (Study # WIL-12377), this study used dose levels ranging from 10 to 1000 mg/kg/day. The comparable doses between the 2 studies were the 50, 100 and 150 mg/kg/day, based on the effects noted in the range-finding study at 150 mg/kg/day which included clinical signs and transient body weight decrease, a supportable Maternal Toxicity NOAEL of 10 mg/kg/day can be established.

Uncertainty Factor (UF): 100X (10X for inter-species extrapolation and 10X for intra-species variability for short and intermediate term exposure scenarios, and 1000X (additional 10X for extrapolation from using a short term study for long term exposure) for long term exposure scenarios.

Comments about Study/Endpoint and Uncertainty Factor: The endpoint selected is adequate for these risk assessment scenarios. A 28-day dermal toxicity study is available; however, it is classified as unacceptable guideline with numerous deficiencies and cannot be used for regulatory purposes.

3.4.3 Inhalation Exposure (Short, Intermediate and Long Term)

The critical effects of furfural exposure *via* the inhalation route are the histopathological changes noted in the respiratory and olfactory epithelium of the nasal cavity reported in the subchronic

inhalation toxicity study in rats. In evaluating the risks that a compound may pose to human health after exposure *via* the inhalation route, different methodologies have been historically used by the USEPA. The Agency's current approach to calculating risks due to inhalation exposure is based on the guidance methodology developed by the Office of Research and Development (ORD) for the derivation of inhalation reference concentrations (RfCs) and human equivalent concentrations (HECs) for use in margin of exposure (MOE) calculations. Under this approach, endpoint selection is based on the endpoints occurring at the lowest HECs (which may or may not be the lowest animal NOAEL).

For all exposure periods:

Study Selected: Subchronic Inhalation Toxicity - Rat; OPPTS 870.3465 [§82-4]; OECD 413.

MRID. No.: 46426504 and 46426505

Executive Summary:

In a subchronic inhalation toxicity study (MRID 46426504 and 46426505), furfural (99% a.i.) commercially obtained from Sigma/Aldrich, Brussels, was administered as a vapor by the nose-only inhalation route to 5 rats/sex/group (Fischer F344 strain) initially to concentrations 0, 40, 80, 160, 320, 640, and 1280 mg/cu.m.for 6 hours per day, 5 days per week for 28 weeks. These dose groups were designated as Groups A to G, respectively. Additional treatment groups exposed to periods of 3 hours/day (5/sex/group) were exposed to furfural vapors at 320, 640 and 1280 mg/cu.m., 5 days per week for 28 days and were designated as groups H, I and J, respectively. Because of excessive mortalities in groups F, G and J (640 and 1280 mg/cu.m.), the study design was changed. Group F (640 mg/cu.m.) was discontinued and two new groups with fresh animals were set up: 20 mg/cu.m. for a 6 hour exposure, 5 days per weeks for 28 days and designated as group G2 and 160 mg/cu.m. for 3 hour exposure periods, 5 days per weeks for 28 days designated as group J2.

Additional groups of rats (5/sex/group) were dosed by gavage with furfural dissolved in corn oil daily for 28 days to provide a toxicity comparison between the oral and inhalation routes of exposure over the same period of time. This DER focuses primarily on the inhalation treatments. Only partial detail on the oral experiments was provided in the study report (MRID 46426505) and therefore, the oral studies are only presented in brief summary detail.

The inhalation treatment groups were evaluated daily for toxicity, weekly for body weight and food consumption, and terminally for hematology changes, clinical chemistry, gross and histopathological observations.

Group F (640 mg/cu.m.) was dropped after deaths occurred during at days 1 and 8. All animals exposed to concentrations of 1280 mg/cu.m. whether for 6 hours (Group G) or for 3 hours, Group J, died in the first day of exposure. These groups were replaced using lower

concentrations and designated G2 and J2 as noted above. There were no more mortalities in the revised dosing treatments for the rest of the study.

Body weight, food consumption, and clinical pathology were not adversely affected by the inhalation treatments. Pathological changes were seen in the nasal epithelium, some affecting all animals at all treatment levels. Other effects were generally dose related.

Treatment related pathological effects were limited to olfactory and respiratory epithelium of the nasal cavity. There were no treatment related effects noted in the kidney, liver, spleen and thymus. Respiratory epithelial atypical hyperplasia was seen in all treated males and females (5/5) for 6 hour exposure groups 20 mg/cu.m. to 320 mg/cu.m. (Groups G2, B, C, D, and E) and 3 hour exposure groups of 160 mg/cu.m to 640 mg/cu.m. (Groups J2, H and I). Respiratory epithelial squamous metaplasia was also found in all males and female (5/5) for the same 6 hour exposure groups (G2, B, C, D and E) and all of the females (5/5) for the 3 hour exposure groups (J2, H and I) and 3-4/5 males in the same 3 hour exposure groups. Respiratory epithelial squamous metaplasia and atypical hyperplasia were seen in males and females in a suggestive dose-response from the lowest concentrations the higher ones. Thus there were no dosed groups where inhalation did not result in nasal epithelium damage, however, the damage was less severe in the 3 hour exposure groups compared to the 6 hour exposure groups of animals.

The Systemic Toxicity LOAEL is equal to or less than 20 mg/cu.m. (lowest dose tested) based on nasal epithelial pathology seen throughout all of the treated animal groups. There was no Systemic Toxicity NOAEL identified (less than 20 mg/cu.m).

Dose and Endpoint used for risk assessment: LOAEL = 20 mg/cu.m. based on nasal epithelial pathology seen throughout all of the treated animal groups. No NOAEL was established. The human equivalency concentrations (HECs) are presented in Table 3. Refer to Appendix A for explanation of the methodology and calculations.

Uncertainty Factor (UF): 300X (3X for inter-species extrapolation, 10X for intra-species variability and 10X for an extrapolation from a LOAEL to NOAEL) for short and intermediate term exposure scenarios and 1000X for long term exposure scenarios (10X for use of a short term study for long term exposure scenarios).

Comments about Study/Endpoint and Uncertainty Factor: The endpoint selected is appropriate for these risk assessment scenarios since it is an inhalation toxicity study.

Table 3: Summary of Inhalation Toxicological Doses and Endpoints Selected Using the RfC Methodology

Relevant Study		LOAEL (mg/m^3)	NOAEL (mg/m^3)	Da	Dh	Wa	Wh	RGDR*	HEC (mg/m^3)	inter	Intra	UF[§]
HEC Array for Non-Occupational Risk Assessment												
Acute Exposure												
28-Day Inhalation Study - RATS	Extrathoracic region (6hr exp.)	20	N.A.	6	24	1	1	0.115	**0.58**	3	10	10
	Extrathoracic region (3hr exp.)	160	N.A.	3	24	1	1	0.115	2.30	3	10	10
Short-, Intermediate-, and Long-term Exposure												
28-Day Inhalation Study - RATS	Extrathoracic region (6hr exp.)	20	N.A.	6	24	5	7	0.115	**0.41**	3	10	10
	Extrathoracic region (3hr exp.)	160	N.A.	3	24	5	7	0.115	1.64	3	10	10
HEC Array for Occupational Risk Assessment												
Acute Exposure												
28-Day Inhalation Study - RATS	Extrathoracic region (6hr exp.)	20	N.A.	6	8	1	1	0.115	**1.73**	3	10	10
	Extrathoracic region (3hr exp.)	160	N.A.	3	8	1	1	0.115	6.90	3	10	10
Short-, Intermediate-, and Long-term Exposure												
28-Day Inhalation Study - RATS	Extrathoracic region (6hr exp.)	20	N.A.	6	8	5	5	0.115	**1.73**	3	10	10
	Extrathoracic region (3hr exp.)	160	N.A.	3	8	5	5	0.115	6.90	3	10	10

[§] 10X UF retained for LOAEL to NOAEL extrapolation.

* Input parameters for the derivation of RGDRs were obtained from "Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry" (USEPA, 1994) Tables 4-4, 4-5, and 4-6.

3.4.4 Margins of Exposure

Summary of the levels of concern for Margins of Exposure (MOEs) for risk assessment.

Route / Duration	Short-Term (1-30 Days)	Intermediate-Term (1 - 6 Months)	Long-Term (> 6 Months)
Occupational (Worker) Exposure			
Dermal	100	100	1000
Inhalation	300	300	3000
Residential (Non-Dietary) Exposure			
Oral	NA	NA	NA
Dermal	100	100	1000
Inhalation	300	300	3000

For **occupational and residential (bystander) short- and intermediate-term dermal** exposure risk assessments, the LOC is for MOEs of 100 or less. This is based on the conventional 100X uncertainty factor, which includes the 10X for intra-species extrapolation and 10X for inter-species variation. For long-term exposure assessments, the LOC is for MOEs of 1000 or less (additional 10X for use of a short-term study for long-term exposure assessments).

For **occupational and residential (bystander) short- and intermediate-term inhalation** exposure risk assessments, the LOC is for MOEs of 300 or less. This is based on uncertainty factors of 10X for intra-species extrapolation, 3X for inter-species variation and a additional 10X for extrapolation from a LOAEL to a NOAEL. For long term exposure assessments, the LOC is for MOEs of 3000 or less (additional 10X for use of a short term study for long term exposure assessments).

3.4.5 Recommendation for Aggregate Exposure Risk Assessments

There is not a common effect observed in the studies selected to assess dermal and inhalation exposure; therefore, aggregation of risk from these two routes is not appropriate.

3.4.6 Classification of Carcinogenic Potential

The carcinogenic potential was not classified at this time as the available long-term data was limited to open literature reports. However, the NTP gavage study produced dose related mortality and centrilobular necrosis and cystic degeneration but no significant increases in cancer. The NTP gavage study in mice produced multifocal necrosis in liver and increased incidence of cholangiocarcinoma and biliary dysplasia which was viewed as a minimal focus for carcinogenesis. Available open literature mutagenicity studies were inconclusive. The carcinogenic potential may be reconsidered if additional long-term data are submitted.

The doses and toxicological endpoints selected for various exposure scenarios that were discussed previously are summarized in Table 4.

Table 4. Summary of Toxicological Doses and Endpoints for Chemical for Use in Human Risk Assessments

Exposure Scenario	Dose Used in Risk Assessment, UF	FQPA SF* and Level of Concern for Risk Assessment	Study and Toxicological Effects
Dermal Short-Term (1 - 30 days)	NOAEL = 10 mg/kg/day, UF 100	N/A	Prenatal developmental in rats; **Maternal** LOAEL = 50 mg/kg/day based on clinical signs.
Dermal Intermediate-Term (1 - 6 months)	NOAEL = 10 mg/kg/day, UF 100	N/A	Prenatal developmental in rats; **Maternal** LOAEL = 50 mg/kg/day based on clinical signs.
Dermal Long-Term (> 6 months)	NOAEL = 10 mg/kg/day, UF 1000 (extra 10X for extrapolation for duration)	N/A	Prenatal developmental in rats; **Maternal** LOAEL = 50 mg/kg/day based on clinical signs.
Inhalation All Durations	Refer to Table 3 for the HEC Array for Bystander and Occupational Exposure	N/A	28-day inhalation toxicity in rats; LOAEL = 20 mg/ cu.m. nasal epithelial pathology seen throughout all of the treated animal groups, no NOAEL was identified

UF = uncertainty factor, FQPA SF = FQPA safety factor, NOAEL = no observed adverse effect level, LOAEL = lowest observed adverse effect level, PAD = population adjusted dose (a = acute, c = chronic) RfD = reference dose, MOE = margin of exposure, LOC = level of concern, NA = Not Applicable

* Refer to Section 3.5

3.5 FQPA Safety Factor

Furfural is a new a.i. proposed for greenhouse ornamentals which is considered to be a 'non-food use' and is not subject to the amendments to the Federal Food, Drug, and Cosmetic Act (FFDCA) promulgated under the Food Quality Protection Act (FQPA) of 1996, and an aggregate risk assessment is not required.

3.6 Endocrine disruption

Following the recommendations of its Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), EPA determined that there was scientific basis for including, as part of the endocrine disruption screening program, the androgen and thyroid hormone systems, in addition to the estrogen hormone system. EPA also adopted EDSTAC's recommendation that the Program include evaluations of potential effects in wildlife. As the science develops and resources allow, screening of additional hormone systems may be added to the Endocrine Disruptor Screening Program (EDSP).

4.0 EXPOSURE ASSESSMENT

4.1 Summary of Proposed Uses

Furfural is a new active ingredient proposed as a fumigant to control root infesting plant parasitic nematodes, and fungal plant diseases in greenhouse soil used for growing ornamentals and other non-food commodities. In this action, the end-use product containing 90% furfural in a liquid formulation (MULTIGUARD^{TM} PROTECT) is proposed for use in growing media and/or soils in greenhouses for cut flowers, cut greens, transplants, propagative materials, ornamentals and other non-food/non-feed commodities. This use was previously assessed in D316219 (K. O'Rourke, 10/13/05). This revised assessment reflects the following new information provided by the registrant, including: changes to the proposed label indicating a reduction in the pre-plant application rate (from 540 lb ai/A to 45 lb ai/A, now equal to the post-plant application rate), and a prohibition of the use of sterile soil/growing media; new dislodgeable foliar residue data; and industry practices regarding air exchange rates used in greenhouses.

The proposed label states that applications may be made via broadcast surface spray (handgun), through overhead irrigation, through drip irrigation, or back-pack sprayer. The treated area should be watered in after application with 125 gallons of water per 1000 ft^2. The recommended treatment interval is 14 to 28 days, for 4 to 8 applications per crop.

For drench applications, the proposed label states that the solution should be applied until it begins to drip through the bottom of the pots, with applications made on a 7- to 28- day schedule throughout the growing season.

4.2 Dietary Exposure/Risk Pathway

Furfural is a new active ingredient; therefore, there are no existing tolerances for this chemical. The proposed use is for greenhouse ornamentals. Because this is a non-food use, the "food only" portion of the dietary assessment is not applicable.

The proposed use pattern for furfural is considered to have minimal potential for causing drinking water contamination (e.g., the pre-plant application requires 5 gallons of 90% furfural formulation, mixed with 45 gallons of water, to be applied per acre, and then to be watered-in with at least 5,445 gallons per acre). A dietary exposure assessment for drinking water was not conducted.

4.3 Residential/Bystander Exposure

Reference: *Furfural: Revised Occupational and Residential Risk Assessment to Support Request for Registration of Furfural in Greenhouses; PC Code: 043301.* C. Smith, D331184, 7/27/06 (Attachment 1)

Data Evaluation Report on the Laboratory Volatility of Furfural from Soil [MRID 46106301]. J. Melendez (EFED), D298145 (Attachment 2)

Residential uses are not proposed for furfural, however, non-occupational bystander exposure to furfural may occur because of emissions from treated greenhouses. These emissions can travel to non-target areas which could lead to negative impacts on human health, and will be referred to simply as bystander risks in this assessment. To evaluate the potential risks to bystanders from greenhouse applications, HED's Fumigant Team has developed methodologies for calculating potential exposure associated with fumigant use.

HED used ISCST3 or the Industrial Source Complex: Short-Term Model to develop risk estimates for bystanders associated with furfural greenhouse uses (http://www.epa.gov/scram001/). [Note: Also refer to http://www.epa.gov/scram001/guidance/guide/appw_03.pdf for additional information concerning the development and validation of ISCST3.] The ISCST3 modeling method uses the Agency developed, Industrial Source Complex Short Term (ISCST3) model (which is used to determine a key ISCST3 input parameter known as flux - i.e., the numerical means to quantify emission rates from a treated field, building or structure) to model the range of concentrations which might be found under different conditions of application rate, weather, source size (e.g., greenhouse size), and distance from the greenhouse.

The greenhouse industry is extremely varied and commercial operations can range from small sole proprietors to large scale commercial propagation and production facilities. The nature of their products are also quite varied which causes them to prepare soil media for use in many ways. Considering this information, along with the California Department of Pesticide Regulation's (CDPR) existing permit conditions for greenhouse applications for methyl bromide, EPA modeled emissions of furfural for greenhouse applications. The CDPR permit conditions for the greenhouse use can be found in:

- Andrews, C. 1994. ***Suggested Permit Conditions for Methyl Bromide Soil Fumigation Within a Greenhouse.*** CDPR. ENF 94-017. May 2, 1994 [Note: Additional information can also be found at http://www.cdpr.ca.gov/docs/county/training/inspprcd/ipmanual.pdf]

Besides utilizing the CDPR permit conditions in the greenhouse ISCST3 analyses, one of the most important parameters for ISCST3 that must be determined is the flux, or rate of pesticide emissions from the treated fields, buildings or structures per unit area per unit time. In essence, flux represents how quickly the pesticide moves or volatilizes into the surrounding atmosphere.

In order to run the ISCST3 model for furfural, it was necessary to estimate the furfural flux rate. Furfural has no field volatility studies that quantify furfural emissions from treated greenhouses to use to calculate flux. A laboratory soil volatility study was provided for furfural, however, there are significant concerns regarding its applicability to the volatility profile of furfural when it is applied in greenhouses. When used for this purpose, the study limitations include:

- the study was conducted in a controlled laboratory environment, which may not reflect the air movement, temperature, humidity, and sunlight differences that occur in a greenhouse;
- the results are based on only one sample, and one type of soil (collected from North Dakota - which is may not be representative of greenhouses throughout the United States); and
- there was approximately a 25-fold difference between the amount of furfural released from sterile and viable soil in the first day (i.e., 5.9% vs. 0.23%); therefore, variation in microbial presence and activity in the soil is a significant factor. The flux rate in this assessment was based on the viable soil measurement (i.e., 0.23%). Note: the registrant has revised the proposed label to prohibit the use of furfural on sterile soil/growing media.

HED estimated a flux rate of 0.5 ug/m^2-s, for pre- and post-plant applications, based on the laboratory soil volatility study. The total amount of furfural emitted during a treatment is proportional to the size of a treated greenhouse, the application rate, and the amount emitted. The area treated for greenhouses is summarized below:

- **Greenhouse Area Treated:** 5,000; 10,000; 20,000; 40,000; 45,000; and 50,000ft^2; and

[Note: Results have only been summarized for the smallest and largest aspects of this range. Results for all area treated values are included in Appendix B of Attachment 1. The amounts treated were also based on values included in the permit condition documents referenced above.]

A broad range of meteorological conditions were considered in the ISCST3 analysis completed for greenhouses in order to evaluate the range of risks that might be anticipated under actual weather conditions. These include:

- **Windspeed:** 1 to 4.5 meters/second (2.3 to 10 mph); and

- **Stability Class:** B, C, and D (B is least stable, D is most stable atmosphere). Atmospheric stability is a measure of how turbulent the atmosphere is at any given time.

The results of the bystander assessment are summarized in Table 5. [Note: CDPR has developed a number of permit conditions using ISCST3 and the conditions generally used are 1.4 m/s and stability class C.]

Table 5. MOEs Estimated for Various Distances Downwind For Greenhouse Fumigations Using the EPA ISCST3 Method Based On LOAEL HEC And UF = 300

Application Type	Amount of Treated Soil	Distance (M)	MOEs For Differing Meteorological Conditions At Specified Distances [1]									
			1 m/s 2.3 mph	1.4 m/s 3.1 mph	1.8 m/s 4 mph	2.2 m/s 5 mph	2.7 m/s 6 mph	3.1 m/s 7 mph	3.6 m/s 8 mph	4.0 m/s 9 mph	4.5 m/s 10 mph	4.5 m/s 10 mph
			Stab D	Stab C	Stab C	Stab C	Stab C	Stab C	Stab C	Stab C	Stab C	Stab B
Post-plant	5,000 sq ft	2	90	150	190	240	290	330	390	430	480	590
		30	180	**360**	460	560	680	790	910	1000	1100	1800
		50	240	560	720	870	1100	1200	1400	1600	1800	3300
		100	460	1400	1800	2100	2600	3000	3500	3900	4400	9000
		300	2400	8200	11000	13000	16000	18000	21000	24000	26000	59000
		500	5400	20000	26000	32000	39000	45000	52000	58000	65000	160000
		1000	17000	70000	90000	110000	140000	160000	180000	200000	230000	600000
	50,000 sq ft	2	45	79	100	130	150	180	200	230	260	320
		30	72	140	190	230	280	320	370	410	460	640
		50	88	190	240	290	360	410	480	530	600	860
		100	130	**300**	380	470	570	660	770	850	960	1600
		300	340	1000	1300	1600	2000	2300	2700	3000	3400	7100
		500	650	2300	3000	3600	4400	5100	5900	6600	7400	17000
		1000	1900	7400	9500	12000	14000	16000	19000	21000	24000	63000

[1] Acute bystander MOEs were calculated using an HEC of 580 $\mu g/m^3$, where an MOE of 300 or more does not exceed HED's level of concern.

The bystander exposures of concern are those outside the greenhouse which may occur in the general population to those living in proximity to a facility. As mentioned previously, the inhalation LOC for bystanders is an MOE of 300 or greater, below which indicates a risk of concern. For small greenhouse fumigation scenarios, risks are not of concern at distances greater than 30 meters downwind, and at distances greater than 100 meters for larger greenhouses.

5.0 AGGREGATE RISK ASSESSMENTS and RISK CHARACTERIZATION

As mentioned previously, furfural is a new active ingredient for which there are no existing tolerances, and the proposed use is for greenhouse ornamentals. For non-food uses, where there are no food residue data or tolerances, an aggregate risk assessment is not required under the FQPA, and therefore, was not conducted.

6.0 CUMULATIVE RISK

Unlike other pesticides for which EPA has followed a cumulative risk approach based on a common mechanism of toxicity, EPA has not made a common mechanism of toxicity finding as to furfural and any other substances, and furfural does not appear to produce a toxic metabolite produced by other substances. For the purposes of this registration action, therefore, EPA has not assumed that furfural has a common mechanism of toxicity with other substances. For information regarding EPA's efforts to determine which chemicals have a common mechanism of toxicity and to evaluate the cumulative effects of such chemicals, see the policy statements released by EPA's Office of Pesticide Programs concerning common mechanism determinations and procedures for cumulating effects from substances found to have a common mechanism on EPA's website at http://www.epa.gov/pesticides/cumulative/.

7.0 OCCUPATIONAL EXPOSURE

Reference: *Furfural: Revised Occupational and Residential Risk Assessment to Support Request for Registration of Furfural in Greenhouses; PC Code: 043301.* C. Smith, D331184, 7/27/06 (Attachment 1)

The end-use product containing 90% furfural in a liquid formulation (MULTIGUARD™ PROTECT) is proposed for use in growing media and/or soils in greenhouses. Applications may be made via broadcast surface spray (groundboom, handgun, or back-pack), injection through overhead irrigation, or through drip irrigation, and drench applications. This revised assessment reflects the following new information provided by the registrant, including: changes to the proposed label indicating a reduction in the pre-plant application rate (from 540 lb ai/A to 45 lb ai/A, now equal to the post-plant application rate), and a prohibition of the use of sterile soil/growing media; new dislodgeable foliar residue data; and industry practices regarding air exchange rates used in greenhouses.

7.1 Dermal Handler Exposures and Risks

No chemical-specific data were available to assess potential dermal exposures to handlers from the proposed uses. The dermal exposure assessment for furfural was conducted using dermal unit exposure data available in the Pesticide Handler's Exposure Database (PHED) Surrogate Table (v1.1., 1998). For some of the occupational handler scenarios presented in Table 6 that reflect baseline clothing, dermal occupational handler risks are of concern (i.e., the MOEs do not reach 100). However, when gloves are added, all handler scenarios have MOEs of 100 or greater, and therefore, are not of concern.

Table 6. Dermal Handler Risk Estimates for Proposed Uses of Furfural										
Exposure Scenario	Application Rate [1]	Area Treated Daily [2]	Baseline Dose	Baseline MOE	PPE-G Dose	PPE-G MOE	PPE-G,DL Dose	PPE-G,DL MOE	Eng Cont Dose	Eng Cont MOE
Mixer/Loader										
Mixing/Loading Liquid Concentrates	45 lb ai/acre	5 acre	9.3	1.1	0.074	140	0.055	180	0.028	360
	0.00814 lb ai/gal	1000 gals	0.34	30	0.0027	3,700	0.002	5,100	0.0010	10,000
Applicator										
Applying Sprays via Groundboom	45 lb ai/acre	5 acre	0.045	220	0.045	220	0.035	280	0.016	620
	0.00814 lb ai/gal	1000 gals	0.0016	6,100	0.0016	6,100	0.0013	7,800	0.00058	17,000
Applying Sprays via Overhead Irrigation	45 lb ai/acre	2 acre	HED has no data to assess the dermal exposure for this use pattern, however, dermal exposure from this scenario is expected to be minimal.							
	0.00814 lb ai/gal	1000 gals								
Applying Sprays via Drip Irrigation Equipment	45 lb ai/acre	2 acre	HED has no data to assess the dermal exposure for this use pattern, however, dermal exposure from this scenario is expected to be minimal.							
	0.00814 lb ai/gal	1000 gals								
Mixer/Loader/Applicator [3]										
Mixing/Loading/Applying Liquids via Handgun Equipment	45 lb ai/acre	2000 ft^2	0.02	490	0.014	700	0.0074	1,400	NF	NF
	0.00814 lb ai/gal	40 gals	0.0032	3,100	0.0022	4,500	0.0012	8,600	NF	NF
Mixing/Loading/Applying Liquids via Low Pressure Handwand	0.00814 lb ai/gal	40 gals	0.47	21	0.002	5,000	0.0017	5,800	NF	NF

NF = Not Feasible
NA = Not Available

[1] 45 lb ai/acre is the maximum application rate for pre- and post-plant applications
0.00814 lb ai/gal is the maximum application rate for drench (potted plant) applications

[2] Amount handled per day values are HED estimates of acreage treated or gallons applied based on Exposure SAC SOP #9 "Standard Values for Daily Acres Treated in Agriculture," industry input, HED estimates, and *data from the California Pesticide Use Survey for 2000* that examined the area of greenhouse soil treated with the soil fumigant methyl bromide.

[3] The label restricts applicators from applying more than 2,000 ft^2 per day when using handheld application equipment

7.2 Inhalation Handler Exposures and Risks

No chemical-specific data were available to assess potential inhalation exposures to handlers from the proposed uses. *Inhalation handler risks for furfural were not assessed using PHED* as furfural is much more volatile (2 mm Hg at 20 °C) than the pesticides that are incorporated into PHED. As a result, inhalation risks would be underestimated if PHED data were used to assess inhalation handler exposures. HED believes that the inhalation postapplication exposures and risks presented in Section 7.4 can be considered a surrogate to represent inhalation handler exposures and risks.

7.3 Dermal Postapplication Exposures and Risks

The registrant submitted a non-guideline dislodgeable foliar residue (DFR) study conducted on poinsettias (MRID# 46809701) for use in assessing potential dermal exposures to postapplication workers from the proposed furfural uses:

> Agriguard Multiguard™ Protect was applied using an eyewash bottle (to simulate applications made with overhead irrigation and backpack sprayers) to poinsettia foliage at one test site in a greenhouse in South Africa. Applications representing a high and low application rate (i.e., 15 and 3.75 oz/20 gal/1,000 ft^2, respectively) were made. Three treated plants for each application rate were sampled in duplicate. Leaf punch samples were collected starting at 30 minutes and up to 72 hours after the final application; no background samples were collected. DFR values were not corrected for field fortification recoveries as all overall field fortification recoveries were >90%. For both application rates, the maximum average DFR values occurred within the first 2 hours after the application (2.8 $\mu g/cm^2$ and 4.4 $\mu g/cm^2$ for the high and low rates, respectively) and declined to approximately 0.7 $\mu g/cm^2$ by 72 hours after application. The average DFR values did not drop below the LOQ (0.1 ppm). First-order dissipation kinetics was assumed in generating dissipation curves. HED estimated furfural half-lifes of 2.4 days (r^2= 0.29) and 2.8 days (r^2= 0.23) for the high and low application rates, respectively, and coefficients of variation for the samples ranged from 6.26 to 78.6 percent.

These data have been reviewed (D328938) and their quality is considered to be poor, however, they have been used in this assessment as surrogate data until the required guideline DFR study is complete. In addition to the DFR data, dermal transfer coefficients from the Science Advisory Council for Exposure Policy Number 3.1: Agricultural Transfer Coefficients, August 2000, were used to estimate dermal exposures during postapplication activities. The assumptions of an 8-hour work day, 70-kg body weight, and 100% dermal absorption were also used in the postapplication exposure assessment.

Risk Summary: The postapplication exposure assessment indicates that dermal occupational risks are of concern (i.e., the MOEs are less than 100) on day 0, and up to 9 days following application, depending on the scenario. Interim restricted entry intervals (REIs) are estimated to be 12 hours for containerized ornamentals, and 9 days for cut flowers. A summary of the results for each crop/activity combination considered for each time-frame is provided in Table 7.

Table 7. Summary of Occupational Postapplication Dermal Risks

Crop Grouping	Application Rate (lbs ai/acre)	Transfer Coefficient	REI	MOE at Day 0
Ornamentals	45	5100 (cut flowers and foliage)	9 days	12
		400 (all other nursery crops)	12 hours	110

7.4 Inhalation Postapplication Exposures and Risks

No chemical-specific data were available to assess potential inhalation exposures to postapplication workers from the proposed furfural uses. In this instance, HED utilized EPA's Multi-Chamber Concentration and Exposure Model (MCCEM) to estimate furfural concentrations inside the greenhouse after a furfural application. Furfural concentrations were modeled assuming air exchange rates ranging from two (average wintertime greenhouse air exchange rate) to ninety (typical summertime rate) per hour. The model also required an emission or flux rate that quantified how quickly furfural moves or volatilizes into the surrounding atmosphere. Numerous factors can influence flux rates such as application rate, type of application, techniques used to control emissions (e.g., tarps, water seals), temperature, wind and weather conditions, soil type, and others.

In order to run the MCCEM model for furfural, it was necessary to estimate the furfural flux rate. Furfural has no field volatility studies that quantify furfural emissions from treated greenhouses to use to calculate flux. HED estimated a flux rate of 0.5 ug/m^2-s from the laboratory soil volatility study discussed previously, in Section 4.3 for residential/bystander exposure. There are significant uncertainties regarding the applicability of this study data to the volatility profile of furfural when it is applied in greenhouses. As such, HED believes that actual postapplication inhalation worker risks may be greater than those presented in Table 8. Risks were calculated for the worst one hour period and the eight hour average (after application). For all greenhouse postapplication exposure scenarios, inhalation postapplication occupational risks are of concern (i.e., the MOEs are less than 300) on day 0 using worst-case air exchange rates. Postapplication inhalation MOEs do not reach 300 until the air exchange rates are increased to 65 per hour (based on 8-hour average) or 90 per hour (based on 1-hour average).

Table 8: Summary of Occupational Postapplication Inhalation Risks

Exposure Scenario	Air Exchanges (hr^{-1}) [1]	Exposure Period	Concentration (mg/m^3)	MOEs [2]
Pre- and Post-plant	2	1 hour max	0.25	7.0
		8 hour avg	0.18	9.4
	6	1 hour max	0.083	21
		8 hour avg	0.062	28
	60	1 hour max	0.0083	210
		8 hour avg	0.0062	280
	65	1 hour max	0.0076	230
		8 hour avg	0.0057	**300**
	80	1 hour max	0.0062	280
		8 hour avg	0.0046	370
	90	1 hour max	0.0055	**310**
		8 hour avg	0.0041	420

[1] The minimum wintertime greenhouse air exchanges are 2 per hour (Buffington et. al 2004); typical summertime rates range from 60 to 90 per hour (comments submitted by registrant from Univ. of MD fact sheet).

[2] Short- and Intermediate-term occupational MOEs were calculated using an HEC of 1.73 mg/m^3, where an MOE of 300 or more does not exceed HED's level of concern.

8.0 DATA NEEDS/LABEL REQUIREMENTS

8.1 Chemistry - None

8.2 Toxicology

- An acceptable 28-day dermal toxicity study
- A guideline 90- or 28-day inhalation study (The previously-required 90-day inhalation study may be reduced to 28 days if it will more closely match the exposure pattern. Although a 28-day inhalation study was submitted, and was used to select an endpoint for this assessment, it was a non-guideline study which lacked the correct number of animals, adequate dosing levels, and number of tissues examined for histopathology).

8.3 Exposure

Data requirements:

- A field/greenhouse volatility study for each major application method (i.e., groundboom, overhead spray, overhead irrigation, and drip irrigation) that measures the flux inside the greenhouse, as well as the outside perimeter. A protocol for this study has recently been submitted and reviewed (D331182); several changes are necessary for the protocol to be acceptable.
- A dislodgeable foliar residue study (or soil residue transfer data if more applicable) to assess postapplication exposure for tasks associated with greenhouse ornamentals. A protocol for this study has recently been submitted and reviewed (D331180); it was found to be acceptable, with recommendations for minor changes.

Label change recommendations:

- The Use caption should be: RESTRICTED USE PESTICIDE; include a statement that furfural is to be used only by a Certified Applicator (or persons under their direct supervision);
- The signal word should be DANGER, (not Warning);
- Include a statement indicating that mixing/loading should be done either outside, or in a well ventilated area, and should be done by or under the direct supervision of a Certified Applicator.

[Note that when the required data have been submitted and reviewed, the following label restrictions will be reconsidered]:

- Increase the required ventilation rate from 12 air changes per hour (ACH) to at least 65 ACH during mixing/loading and application, and for at least 48 hours following application (the laboratory soil volatility study indicates that volatilization plateaus after 2 days);
- Change the reentry statement to "A restricted entry interval (REI) of 12 hours is required for entry into treated areas for containerized ornamentals, and an REI of 9 days is required for cut flowers"; and
- Institute a buffer zone of 30 meters for greenhouse treatments of 5,000 square feet or less, and 100 meters for larger applications.

9.0 ATTACHMENTS

Attachment 1: *Furfural: Revised Occupational and Residential Risk Assessment to Support Request for Registration of Furfural in Greenhouses; PC Code: 043301.* C. Smith, D331184, 7/27/06.

Attachment 2: *Data Evaluation Report on the Laboratory Volatility of Furfural from Soil [MRID 46106301].* J. Melendez (EFED), D298145.

cc without attachments: C. Smith, S. Gross, J. Arthur, S. Dapson, RAB3 Reading File.

APPENDIX A: Methodologies for Inhalation Risk Calculations

The Agency's approach used to calculate risks due to inhalation exposure (to furfural) is based on the guidance methodology developed by the Office of Research and Development (ORD) for the derivation of inhalation reference concentrations (RfCs) and human equivalent concentrations (HECs) for use in margin of exposure (MOE) calculations (RfC methodology). The RfC methodology applies a dosimetric adjustment that takes into consideration not only the differences in ventilation rate (MV) but also the physicochemical properties of the inhaled compound, the type of toxicity observed (*e.g.* systemic vs. port of entry) and the pharmacokinetic (PK) **but not pharmacodynamic** (PD) differences between animals and humans. Based on the RfC guidance (1994), the methodology for RfC derivation is an estimate of the quantitative dose-response assessment of chronic non-cancer toxicity for individual inhaled chemicals and includes dosimetric adjustment to account for the species-specific relationships of exposure concentration to deposited/delivered dose. This adjustment is influenced by the physicochemical properties of the inhaled compound as well as the type of toxicity observed (*e.g.* systemic vs. port of entry), and takes into consideration the PK differences between animals and humans. Though the RfC methodology was developed to estimate toxicity of inhaled chemicals over a lifetime, it can be used for other inhalation exposures (*e.g.* acute and short-term exposures) since the dosimetric adjustment incorporates mechanistic determinants of disposition that can be applied to shorter duration of exposures provided the assumptions underlying the methodology are still valid. These assumptions, in turn, vary depending on the type of toxicity observed and will be discussed later on in this document. Thus the derivation of a HEC for inhaled gases is described by the following equation:

$$HEC = POD_{study} * \frac{D_{\text{animal exposure (hrs / day)}}}{D_{\text{human exposure (hrs / day)}}} * \frac{W_{\text{animal exposure (days / wk)}}}{W_{\text{human exposure (days / wk)}}} * RGDR$$

Where:

POD_{study}: Point of departure identified in the critical toxicology study
$D_{animal\ exposure}$: Duration of animal exposure (hrs/day; days/wk)
$D_{anticipated\ exposure}$: Anticipated human duration of exposure (hrs/day; days/wk)
RGDR: Regional Gas Dose Ratio

For gases eliciting both port of entry and systemic effects, calculations to estimate the inhalation risk to humans are dependent on the regional gas dose ratio (RGDR). In the case of systemic effects, the RGDR is defined as the ratio of the blood:gas partition coefficient of the chemical for the test species to humans ($H_{b/g\ animal}/H_{b/g\ human}$). When this ratio is unknown or when the $H_{b/g\ animal} > H_{b/g\ human}$ a default value of 1.0 is used as the RGDR. This default is based on the observation that for chemicals where partition coefficient data are available in both rats and humans the RGDR value has usually been comparable or slightly higher than 1. Thus, the use of an RGDR of 1 results in a protective calculation of the inhalation risk. Some of the key assumptions fundamental to the use of the RfC methodology to derive a HEC based on systemic effects include:

1) all the concentrations of inhaled gas within the animal's body are periodic with respect to time (*i.e.* periodic steady state - the concentration *vs* time profile is the same for every week). Periodicity must be attained for at least 90% of the exposure.

2) in the respiratory tract, the air, tissue, capillary blood concentration are in equilibrium with respect to each other.
3) systemically, the blood and tissue concentrations are in equilibrium with respect to each other.

In the case of furfural, the physicochemical properties and metabolism data for the compound indicate that these conditions (*i.e.* periodicity and equilibrium between different compartments) will be achieved in a very short period of time. Under these conditions, therefore, the use of the RfC methodology to estimate acute inhalation risk is appropriate.

When the critical toxic effect in a study occurs in the respiratory tract (*i.e* port of entry effects), the RGDR is not related to the blood:gas partition coefficient of the compound but rather the ratio of the minute volume (MV) to the surface area (SA) of the affected region. In these instances, attaining periodicity or equilibrium between the compartments is not critical (since the effect is a function of the direct interaction between the inhaled compound and the affected region in the respiratory tract) and the RGDR may be calculated using the following equation:

$$RGDR = \frac{MV_{animal} / SA_{animal}}{MV_{human} / SA_{human}}$$

Where:

MV_{animal}: Minute volume for the test species (varies depending on body weight)
SA_{animal}: Surface area of the affected region in animals
MV_{human}: Minute volume for humans (default value is 13.8 l/min)
SA_{human}: Surface area of the affected region in humans

The MV_{animal} is calculated using the allometric scaling provided in USEPA (1988a). The equation for calculation of the MV_{animal} is:

$$\ln MV_{animal} = b_0 + b_1 \ln(BW)$$

Where:

$\ln MV_{animal}$: natural logarithm of the minute volume
b_0 : species specific intercept used in the algorithm to calculate minute volumes based on body weight
b_1: species specific coefficient used in the algorithm to calculate minute volumes based on body weight
ln BW: natural logarithm of the body weight (expressed in kg)

The values for the species-specific parameters used to calculate the MV_{animal} based on body weight and the values for the surface areas of various regions of the respiratory tract (extrathoracic, thoracic, and pulmonary) are provided in the EPA document "Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry" (1994).

The magnitude of the UFs applied when the RfC methodology is utilized takes into consideration the PK differences but not the PD differences. Consequently, the UF for interspecies extrapolation may be reduced to 3X (to account for the PD differences) while the UF for intraspecies variation is retained at 10X. Thus, the UF when using the RfC methodology is customarily 30X. However, for furfural, an extra 10X UF is retained for LOAEL to NOAEL extrapolation.

APPENDIX B: Executive Summaries of Studies Not Cited Previously

Subchronic Oral (Gavage) Toxicity Studies in Rats and Mice; Guideline: OPPTS §870.3100; OPP §82-1; EPA MRID# 46011015.

In two independent studies (MRID 46011015) conducted under the U.S. National Toxicology Program (NTP), Furfural (99% a.i.; Lot # Q112979) was administered for up to 13 weeks in corn oil via gavage to 10 F344/N rats/sex/group at nominal dose levels of 0, 11, 22, 45, 90 or 180 mg/kg/day or 10 $B6C3F_1$ mice/sex/group at 0, 75, 150, 300, 600, or 1200 mg/kg/day. The dosages were administered daily 5 days/week at dose volumes of 5 mL/kg in the rats and 10 mL/kg in the mice. Survival, body weight, body weight gain, and organ weight data were provided. Histopathology liver findings were summarized in the text. The stated purpose of the studies was to evaluate cumulative toxic effects of furfural and to determine the doses to be used in the carcinogenicity studies.

In the rat study, 9/10 males and 10/10 females in the 180 mg/kg group, and 1/10 males and 4/10 females in the 90 mg/kg group died before the end of the study. The majority of the 90 mg/kg deaths were due to gavage injury. Mean body weights and body weight gains were similar to controls; terminal body weights were only slightly increased (p less than or equal to 0.05) in the 45 and 90 mg/kg males compared to controls. In the 90 mg/kg male rats, increases (p less than or equal to 0.05) in absolute and relative (to body) liver weights were observed. A non-dose dependent increase in the incidence of minimal to mild hepatocyte cytoplasmic vacuolization was observed in controls and all treated males (9-10/10 treated vs 4/10 controls). Based on this study, the NTP selected 60 mg/kg/day as the high dose and 30 mg/kg/day as the low dose for the subsequent two year rat study.

The Systemic Toxicity NOAEL is 45 mg/kg/day and the Systemic Toxicity LOAEL is 90 mg/kg/day based on liver weight changes and liver pathological observations. The observation data available in this study for endpoint determination was minimal, this study was used as a range-finding study for the NTP carcinogenesis study.

In the mouse study, all animals that received 1200 mg/kg and the majority of the 600 mg/kg group died within the first few weeks of the study. These deaths were considered treatment-related. At 150 and 300 mg/kg, mean body weights, body weight gains, and terminal body weights were slightly decreased in the males and were similar to controls in the females. Increased (p less than or equal to 0.05) relative (to body) liver weights were observed in the 300 mg/kg males and the 75, 150, and 300 mg/kg females. It was stated that centrilobular hepatocyte coagulative necrosis was observed in the 1200 mg/kg group (8/10 males and 2/10 females) and in males at 600 mg/kg (9/10), 300 mg/kg (1/10), and 150 mg/kg (1/10). Inflammation, characterized by a minimal to mild mononuclear inflammatory cell infiltrate, was also observed in the presence of liver necrosis. Based on this study, the NTP selected 175 mg/kg/day as the high dose and 50 mg/kg/day as the low dose for the subsequent mouse carcinogenicity study.

The Systemic Toxicity NOAEL is less 75 mg/kg/day and the Systemic Toxicity LOAEL is equal to or greater than 75 mg/kg/day based on liver weight changes and liver pathological

observations. The observation data available in this study for endpoint determination were minimal, this study was used as a range-finding study for the NTP carcinogenesis study.

These studies do not completely satisfy the guideline recommendations for a subchronic oral toxicity study in rodents (OPPTS §870.3100; OPP §82-1); however, the data are supportable for use in the choice of regulatory endpoints with appropriate uncertainty factors. These studies were used as range-finding studies for the NTP carcinogenesis studies.

MRID # 46465501: 28-Day Dermal Toxicity - Rats; OPPTS 870.3200 [82-2] (rodent); OECD 410.

Bhoite, P.Y. (2004) Repeated Dose 28-Day Dermal Toxicity Study of Furfural in Rats Followed by a 4-Week Recovery Period. Jai Research Foundation, Department of Toxicology, Gurat, India.. Study Number 4700, December 03, 2004. .

In a 28-day dermal toxicity study (MRID 46465501), technical liquid furfural (98.48% a.i), batch labeled as Dec. 2003, was applied to the shaved skin of Wistar rats (10/sex/dose) at dose levels of 0, 25, 50 and 100 mg/kg bw/day, 6 hours/day, 5 days/week during a 28-day period. The controls animals received applications of water only. These treatment animals were designated as G1, G2, G3 and G4 respectively. Two additional groups of animals (10/sex/group designated as control (G5) and high dose (G6) were treated with water or furfural during the first 4 weeks of with the treatment groups but were also retained for a 4 week post-treatment recovery period. without further treatments.

All rats were observed twice daily for toxicity and weekly for body weight and food consumption. All groups were evaluated for behavioral toxicity prior to treatment and weekly thereafter to the end of their respective treatment periods. Groups G1-4 were assessed during the the 4th week of treatment for clinical pathology (clinical chemistry, hematology and urinalyses), groups G5 and G6, during the 4th week of the recovery period. Ophthalmological examinations were performed on all rats before commencement of treatments and prior to sacrifice. At the end of the 4 week treatment period, groups G1-G4 underwent pathological examination for organ weight changes, gross pathology and histopathological evaluation.

There were no mortalities in any of the groups, no adverse effects on body weight or food consumption; nor were there effects seen in clinical pathology or ophthalmological assessments. There were no treatment related changes in organ weights, gross pathology or histopathological changes. Skin samples apparently were not obtained for histopathology.

Female rats dosed at 100 mg/kg (in both the G4 and G6 treatment groups) showed treatment related effects of drowsiness, dyspnea, clonic convulsion, hyperactivity, tremor, vocalization 3-4 hours post dosing during the first to third. These changes were not dose related or supported by weekly observations made during the four week treatment or recovery periods. The investigating laboratory carried out neurobehavioral observations without providing historical

control information to show that the laboratory had previous experience in performing neurobehavioral assessment of rats.

There were no clear cut adverse effects at the high dose level (100 mg/kg) which were supported by the results (there were no consistent clinical signs, clinical pathology and histopathological of toxicity which were seen in other studies in which furfural was administered at frankly toxic doses and which could have been seen here if the dose was high enough. The high dose levels were far below any limit dose (1000 mg/kg) which could be cited as an acceptable NOAEL if used in the study.

An LOAEL was not achieved in this study, and therefore also lacked a NOAEL. Aluminum foil was used to enclose the furfural liquid on the dermal application site of the rat, this is not an acceptable method.

CONCLUSIONS. This 28-day dermal toxicity study in the rat is unacceptable guideline study and does not satisfy the guideline requirement for a 28-day dermal toxicity study (OPPTS 870.3200 ; OECD 410) in the rat.

The acute study summaries presented below are based on the data evaluation reports (DERs) completed by the Registration division, and expanded to include reported clinical signs:

MRID 46011009. oral study (OPPTS 870.1100) in the rat.

Rana, M. D. (2002). Acute Oral Toxicity Study of Furfural in Rats. Jai Research Foundation, Department of Toxicology, Valvada, Valsad, Gujrat, India. Study No. 3884 dated 10-24-02.

In an acute oral toxicity study (MRID 46011009), 10 male and 10 female Wistar rats (Mean wt range: male 230-242 g, female; 174-175 g, Source: Breeding facility Jai Research Foundation) were given a single dose at graded levels of 70 or 100 or 120 or 140 or 205 mg/kg. Evaluation parameters included signs of gross toxicity and mortality for a subsequent period of 14 days. Initial and weekly body weights, and necropsy findings were recorded on all animals.

Oral LD_{50} Male rats was >100 mg/kg bw
Female rats was > 105 mg/kg bw
Combined dose was> 102 mg/kg

Furfural is of **moderate Toxicity** based on the LD_{50} in male and female rats, EPA Toxicity Category II.

B. <u>Clinical observations</u> - Clinical signs (in general) included lethargy, tremors, abdominal breathing, tachypnea, exophthalmos and piloerection. There were bronchial rales in 100 and 400 mg/kg dose groups. Body weights were not affected in survivors.

C. Gross Necropsy - Decedent animals showed red lungs with hemorrhages, edema. There was mucus exudation in the intestine. Terminal animals showed no significant test related lesions.

MRID 46011010. Acute Dermal Toxicity- Wistar Rat; OPPTS 870-1200; OECD 402

Joseph, S. A. (2003). Acute Dermal Toxicity Study of Furfural in Rats. Jai Research Foundation, Department of Toxicology, Valvada, Valsad, Gujrat, India. Study No. 3950 dated 5-23-03.

In an acute dermal toxicity study (MRID 46011010), Wistar rats, 5/sex (Wt. males 217-263 g, females 201-230 g, Source: Breeding Facility, Jay Res. Foundation) were dermally exposed to Furfural at 145 or 171 or 202 mg/kg bw (dose volume mL/kg). Test sites (10% body surface area) were covered with a gauze and a plastic wrap for 24 hours. Animals were then observed for 14 days. Terminal necropsy was performed.

Dermal LD_{50} Males=192 mg/kg bw / Females = 192 mg/kg bw / Combined = 192mg/kg bw furfural is of **high toxicity** based on the LD_{50} in rats (males / females). The compound is classified as EPA Toxicity Category I.

B. Clinical observations - Lethargy, abdominal breathing and nasal discharge was noted in a few animals.

C. Gross Necropsy - Decedent animals showed froth in trachea, congestion of lungs and hemorrhage/edema, enlarged spleen, petechia in thymus, distended urinary bladder, and hydrometra (uterus).

MRID 46011012. Primary Eye Irritation-NZW Rabbits; OPPTS 870.2400; OECD 405, Clear light yellow liquid.

Joseph, S. A. (2003). Acute Eye Irritation Study of Furfural in Rabbits. Jai Research Foundation. Department of Toxicology, Valvada, Valsad, Gujrat, India. Study No. 3952 dated 5-23-03.

The primary eye irritation potential of Furfural Technical (99.7%) was evaluated in a study in rabbits (MRID 46011012). The test substance (0.1 mL) was instilled into the conjunctival sac of one eye of each of 3 NZW rabbits (Source: Breeding Facility JRF). The other eye served as the control. Ocular irritation was evaluated for 14 days. .

In this study Furfural Technical is a **severe irritant** to the rabbit eyes. The test substance has EPA Toxicity Category II.

A. Observations - (Table 1) Rabbits showed ocular irritation (conrneal opacity, iritis and conjunctivitis) which subsided by 14^{th} day.

MRID 46011013. Primary Dermal Irritation - NZW Rabbit; OPPTS 870.2500; OECD 404

Joseph, S. A. (2003). Acute Dermal Irritation Study of Furfural in Rabbits. Jai Research Foundation, Department of Toxicology, Valvada, Valsad, Gujrat, India. Study No. 3951 dated 5-23-03.

In a primary dermal irritation study (MRID 4601113), 3 young adult NZW rabbits (Source: Breeding Facility JRF) were dermally exposed to 0.5 mL dose of Furfural Technical (99.7%) for 4 hours. The test patches were applied to the dorsal part of clipped surface of the body. The first rabbit was treated in a progressive dose to assure the compound is not corrosive. Animals were observed for 14 days. Dermal Irritation was scored by Draize Method.

In this study Furfural Technical is **slightly irritating** to the rabbit skin. It meets EPA Toxicity Category IV.

Observations - At 72 hours very slight erythema was observed in one rabbit, and well defined erythema and very slight edema in 2 rabbits. The product is a mild irritant.

MRID 46011014. Dermal Sensitization - Guinea Pig; OPPTS 870.2600; OECD 406, 429

Joseph, S. A. (2003). Dermal Sensitization Study of Furfural in Guinea Pigs. JAI Research Foundation, Department of Toxicology, Valvada, Valsad, Gujrat, India. Study No. 3953 dated 5-23-03.

A Maximization study (MRID 46011014) was conducted to assess the sensitization potential of Furfural Technical in guinea pigs. Twenty test and 10 control guinea pigs (Mahaveera Enterprizes, Hyderabad, India) were selected for the study. A 5% concentration of propylene glycol was selected for intradermal injection, Undiluted Furfural was selected (0.2 mL) for topical. A 25% (0.2mL) Furfural in acetone was selected for challenge (topical). Animals were evaluated at 24 and 48 hours after challenge (Report page 10).

In this study, Furfural Technical **is not a dermal sensitizer.**

The following submission was also considered in the development of the risk assessment:

- **MRID 46426506:** "Opinion of EFS Scientific Panel on Food Additives, Flavorings, Processing Aids and Materials in Contact with Food. Authors (Panel Members) Anto, R, Barlow, S, Boskou, e., et al. Dated June 2, 2004. Performing facility European Food Safety Authority (EFS). Study ID No. FT-12-2004-2 (40 pages)"

MUTAGENICITY STUDIES:

MRID 46011017: Bacterial system, *e.g.*, *Salmonella*/mammalian activation gene mutation assay; OPPTS 870.5100 [84-2]; OECD 471, 472.

CITATION: Haddouk, H. (1999). Furfural: Bacterial Reverse Mutation Test, performed at Centre International de Toxicologie, Miserey - 27005 Evreux (FRANCE). Laboratory Study No. 18384 MMO, 8 June 1999. MRID 46011017. Unpublished.

EXECUTIVE SUMMARY: In replicate bacterial reverse mutation assays (MRID 46011017), 5 histidine-deficient (*his*$^-$) strains of *Salmonella typhimurium* (TA1535, TA1537, TA98, TA100 and TA102) were exposed by the direct plate incorporation assay and its preincubation modification (60 minutes) to furfural (Batch No. "02/02/99", 99.94% a.i. dissolved in dimethyl sulfoxide, DMSO) at 5 concentrations ranging from 312.5 to 5000 *u*g/plate, in the presence (+S9) and absence (-S9) of a metabolic activation system prepared from the liver microsomal fraction of rats induced with Aroclor 1254. The number of histidine revertants (*his*$^+$) in test cultures was compared to solvent (negative) control values. In addition to cultures exposed to DMSO (solvent control), other cultures were treated with strain-specific mutagens, to serve as positive controls.

A preliminary dose-ranging cytotoxicity test was carried out at 6 concentrations ranging from 10 to 5000 *u*g/plate ± S9.

In the main mutagenicity assays, slight cytotoxicity was found at the highest concentration, 5000 *u*g/plate for strains TA1537 and TA100, but at no dose was an increase in histidine revertants (*his*$^+$) observed, either in the presence or absence of metabolic activation. Positive controls responded appropriately with marked increases of revertants.

Therefore, furfural technical is considered non-mutagenic in this battery of *Salmonella typhimurium* strains.

This study is classified as **acceptable/guideline**, and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

MRID 46011018: *In vivo* gene mutation in the bacterial gene, *λlacZ*, incorporated into the genome of treated transgenic mice.

CITATION: Steenwinkel, M.J.S.T. and Krul, C.F.A.M. (2003). *In vivo* Gene Mutation Study by Use of *λlacZ*-Transgenic Mice with Furfural, performed at the Department of Biomolecular Sciences, TNO Nutrition and Food Research; Utretchweg 48/3700 A.J. Zeist (The Netherlands). TNO Project Number: 010.44074 (TNO Study: 3934), dated 01 May 2003. MRID 46011018. Unpublished.

EXECUTIVE SUMMARY: In an *in vivo* gene mutation assay in *λlacZ*-transgenic mice (MRID 46011018), 4 groups of 13 males each were administered furfural technical (Batch No.

02/02/20, 99.9% a.i., dissolved in corn oil) by oral gavage at daily doses of 37.5, 75, 150 and 300 mg/kg for 28 days. An additional group of 13 males received 10 mL/kg/day oral doses of the vehicle for 28 days (served as the negative control), while another group of 8 males received a known mutagen, ethylnitrosourea (ENU, 50 mg/kg/day, in dimethyl sulfoxide, DMSO) intraperitoneally (i.p.) for 5 days (served as positive control). On the 28th day, 3 animals from each of the furfural groups and the negative control were sacrificed to obtain data on hepatotoxicity (positive control animals were not assessed for hepatotoxicity). After an additional untreated period of 34 or 35 days (to permit fixation of mutations), the livers were collected from the remaining animals of each furfural group and the negative control and processed for the determination of mutant frequency.

The furfural dose levels for this study were stated by the investigator to have been selected on the basis of a previous 13-week National Toxicity Program (NTP) toxicity study, from which a NOAEL was calculated at 75 mg/kg/day; thus it was anticipated that administration of 300 mg/kg/day would elicit hepatotoxicity . In order to insure sufficient animals would be available for mutation analysis, two additional animals were allocated as reserves to each test group. There were three treatment-related early deaths in the 300 mg/kg/day group, and one in the 75 mg/kg/day group. Several clinical and histopathological adverse reactions were observed in the survivors at the highest dose, 300 *u*g/mL.

However, the mutant frequencies (MFs) of DNA extracted from mouse hepatic cells were not increased over concurrent or the laboratory background negative control values at any dose level of furfural tested. The positive control group yielded the expected significant increase.

Therefore, furfural in corn oil administered up to levels of clinical toxicity and death is not associated with *in vivo* mutagenicity of liver cells in transgenic (male) mice transfected with *λlacZ*. Although this type of assay has no regulatory guideline (neither in FIFRA nor OECD), its negative conclusion is considered **scientifically acceptable**, based on the use of recognized published methodology and technical proficient procedure.

MRID 46011019: ***In vitro* mammalian chromosomal aberrations and sister chromotid exchanges (SCEs) in Chinese Hamster Ovary (CHO) cells, and SCEs in human lymphocytes; *in vivo* mammalian SCEs and chromosome aberrations in mice and humans.**

CITATION: Katz, A.C. and Eikhoff, J. C. (2003). Furfural - Structural Chromosome Aberrations, reports gathered by the Consulting Firm, TOXCEL, Manasas (VA) for the SPONSOR, May 23, 2003. Sponsor I.D. No.: Furfural 2003-NFG-20. MRID 46011019. Published studies.

EXECUTIVE SUMMARY: This submission ("Volume 20 - Toxicology") contains the following three published articles, plus one abstract, on assaying furfural for *in vitro* and *in vivo* chromosome aberrations (CAB) and sister chromatid exchanges (SCE) at *in vitro* concentrations of 25-800 *u*g/mL or 3.5-14.0 x 10^{5} M, and *in vivo* doses of 0.1-200 mg/kg or 1000 - 4000 ppm.

MRID 46011019, ATTACHMENT 1: Adams, T. B., Doull, J. *et al.* (1997). The FEMA GRAS Assessment of Furfural Used as a Flavor Ingredient. *Fd. Chem. Toxicol.* 35: 739-751.

MRID 46011019, ATTACHMENT 2: Eight studies excerpted from The National Toxicology Program (NTP) study: "Toxicology and carcinogenesis of furfural in F344/N rats and B6C3F$_1$ mice (gavage studies)". Technical Report Series No. 382, National Institutes of Health (NIH) Publication No. 90-2837. III. Results: Genetic Toxicology (p. 42), and APPENDIX H (pp. 181-193). [N.B. The entire NTP Report was submitted by Agriguard as Volume 17 of the registration package.

MRID 46011019, ATTACHMENT 3: Gomez-Arroyo, S. and Souza, V. (1985). *In vitro* and occupational induction of SCEs in human lymphocytes with furfural alcohol and furfural. *Mut. Res. 56*: pp. *233-238.*

MRID 46011019 ATTACHMENT 4: Subramanyam, S., Sailaja, D. and Rathnaprobaha, D. (1989). Genotoxic assay of two dietary furans by some *in vivo* cytogenetic parameters [Abstract]. Environmental and Molecular Mutagenicity 14, Supplement 15, p. 239.

REPORTED RESULTS:

Results of these published studies have been mixed: positives for cytogenetic damage (CAB/SCE) in most mammalian *in vitro* assays when tested at severely cytotoxic levels (however, negative in Ames testing), but negative in mice treated up to adverse (toxic) doses as well as in exposed agricultural field workers.

The study authors provided the following information: Brief extracts from these published submissions follow (their assessment by EPA Reviewers are found below):

MRID 46011019 ATTACHMENT 1 (Adams, *et al.*): The authors, acting as the Expert Panel of the Flavor and Extract Manufacturers' Association (FEMA), have summarized previously published literature on *in vitro* and *in vivo* genotoxicity assays of furfural for structural chromosome aberrations as well as other pharmacological, physiological and toxicological effects. On the basis of the reported negative mutagenicity, FEMA concluded that "the effects of furfural result from a non-genotoxic mechanism of action in which high dose levels are hepatotoxic in male $B_6C_3F_1$ mice, leading to cell proliferation and cell death and, after prolonged exposure, liver tumors". It was, therefore, concluded that furfural may continue its safe use as a flavor ingredient under the GRAS rubric. No original experiments on furfural were included in this publication.

MRID 46011019 - ATTACHMENT 2 (NTP Technical Report): Furfural was tested for carcinogenicity in long-term (chronic) studies with rats and mice, using 99% furfural (from The Quaker Oats Company, Chicago, IL), and direct results of these end-points are available. Included in this Report are previously published results sponsored by NTP on the genotoxicity of this substance.

These studies are part of the NTP protocol and are summarized below:

These studies, which have appeared in peer-reviewed journals, include reverse gene mutations in *Salmonella typhimurium*, forward gene mutations in mouse lymphoma cells, cytogenetic evaluations for chromosome aberrations and sister chromatid exchanges (SCEs) in Chinese hamster ovary (CHO) cells and sex-linked recessive lethal mutations in *Drosophila melanogaster* and are summarized below. It should be noted that all genotoxicity assays were conducted with 97.8% a.i. from Radian Corporation (Austin, TX).

MRID 46011019 - ATTACHMENT 3 (Gomez-Arroyo and Souza): These authors presented original research on the effects of furfural and furfuryl alcohol on SCEs in human lymphocyte cultures, as well as the induction of SCEs in lymphocytes drawn from field workers occupationally exposed to these substances. The concentrations applied *in vitro* were, for furfural: 14×10^{-5}, 7×10^{-5}, and 3.3×10^{-5} M; and, for furfuryl alcohol: 9.9×10^{-3}, 6.6×10^{-3} and 3.3×10^{-3} M.

Higher concentrations reduced mitotic indices; but the levels were not listed.

After assuring that no countervailing factors would weight the results, (*i.e.*, no previous exposure to X-irradiation or chemical agents and drugs; no recent viral infection; intakes of tobacco and alcohol), 6 field workers who would come in contact with these substances by exposure, inhalation or touch, were matched in age, sex and like habits to 6 administrative personnel as controls. Periphoral blood samples were drawn from these two groups [after an unspecified time period]. Both sets of heparized blood were appropriately prepared for microscopic examination of furfural on SCEs (using the FPG technique), and the mitotic spindle. Experiments were replicated once; 200 cells were analyzed for c-mitosis and tetraploidy, and 2000 cells were randomly observed to calculate the mitotic index (MI).

In vitro studies showed that furfural at 3.5 or 7.0×10^{-5}M was a strong inducer of SCEs ($p < 0.001$), unlike furfuryl alcohol, which was less active. Furfural, but not furfuryl alcohol, also damaged the mitotic spindle apparatus, as shown by the induction of a strong c-mitotic effects (similar to the action of colchicine, the classic mitotic poison). It also stimulated cell division, as indicated by significantly increased MIs at 3.5, 7.0 and 14.0×10^{-5}M.

Nevertheless, the analysis of SCEs in workers occupationally exposed to furfural at concentrations from 9545 to 38,180 mg/M^3 showed no significant difference compared to controls.

MRID 46011019 - ATTACHMENT 4 (Subramayam, *et al.*): This abstract (from an oral presentation at the 1988 meeting of the Environmental Mutagen Society) described an evaluation of furfural and 2-methyl furan in *in vivo* cytogenetic assays using "somatic" and "meiotic" tissues and multiple parameter in 8 to 10 week-old Swiss albino mice. An increase in "chromosome mutations" in the somatic system occurred at the highest dose of furfural only, 4000 ppm, from intakes ranging from 1000 to 4000 ppm administered for 5 days. There was no inhibition of spindle proteins found, and no retardation of cell division. No genotoxic effects were found in the "meiotic test system" after 5 weeks administration of either furan, nor any sperm head abnormalities. However, no data were presented.

EPA ASSESSMENTS: The following classifications were made by EPA reviewers:

ATTACHMENT 1: **Unacceptable** for purposes other than required by FIFRA Test Guidelines since the published article is a survey of publications by others, and provided no original experimentation.

ATTACHMENT 2: **Acceptable/Guideline** in providing valid genetic toxicology data, as required by FIFRA Test Guidelines and satisfies the guideline requirements for reverse gene mutations in bacteria, *in vitro* mammalian cell gene mutation and chromosome aberrations, and *in vivo* mammalian bone marrow chromosomal aberration tests. In addition, the *in vivo* sister chromatid exchange assay is **acceptable/guideline**; and the D. melanogoster sex-linked recessive lethal assays are acceptable guideline.

ATTACHMENT 3: **Unacceptable**, in providing valid cytogenetic data, however, did not provide many major criteria required by the FIFRA Test Guidelines, such as purity of experimental substances employed, duration of exposure of human subjects to furfural, *inter alia*.

ATTACHMENT 4: The oral presentation, rather than the abstract provided, might have been acceptable in providing valid cytogenetic data required by the FIFRA Test Guidelines, but too many major essentials were missing (purity, definition of "chromosome mutation", "somatic" and "meiotic" tissue", *inter alia* and no primary data were available). Hence, the study is **unacceptable**.

MRID 46011020: **Other Genotoxicity: DNA Damage/Unscheduled DNA Synthesis in Plasmids (pBR322), Bacteriophage (lambda DNA), Bacteria (*B. subtilis*), F344 Rats and B6C3F$_1$ Mice. 870.5500, 870.5550; OECD 482.**

EXECUTIVE SUMMARY: This submission, by TOXCEL, LLC for AGRIGUARD, (titled: *Volume 21 - Toxicology*), is a series of 9 articles (7 published, one unpublished,, and one abstract) reporting results in bacterial and mammalian assays for DNA damage, unscheduled DNA synthesis and other genotoxicity tests, as follows:

ATTACHMENT 1 : Matsui, S., Yamamoto, R. and Yamada, H. (1989). The *Bacillus subtilis*/Microsome Rec-Assay for the Detection of DNA Damaging Substances Which May Occur in Chlorinated and Ozonated Waters. *Water Sci. Technol.* 21: 875-887.

ATTACHMENT 2: Osawa, T. and Namiki, M. (1982). Mutagen Formation in the Reaction of Nitrite with the Food Components Analogous to Sorbic Acid. *Agric. Biol. Chem.* 46: 2299-2304.

ATTACHMENT 3: Phillips, B.J., Jackman, L.I. *et al.* (1997) Furfural does not induce unscheduled DNA synthesis (UDS) in the *in vivo* rat hepatocyte assay. [ABSTRACT]. Proceedings of the Society of Toxicology Annual Meeting, 1997, Cincinnati (Ohio). N.Y. Academy Press.

ATTACHMENT 4: Hadi, S.M., Rehman, S., and Rehman, A. (1989). Specificity of the Interaction of Furfural with DNA. *Mutat. Res. 225*: 101-106.

ATTACHMENT 5: Uddin, S. and Hadi, S.M. (1995). Reactions of Furfural and Methylfurfural with DNA. *Biochem. Molec. Biol. Intern.* 35: (1) 185-195.

ATTACHMENT 6: Adams, T.B., Doull, J. *et al.* (1997). The FEMA GRAS Assessment of Furfural Used as a Flavor Ingredient. *Fd. Chem. Toxicol.* 35: 739-751.

ATTACHMENT 7: Kahn, Q.A. and Hadi, S.M. (1993). Effect of Furfural on Plasmid DNA *Biochem. Molec. Biol. Intern.* 29(6): 1153-1160.

ATTACHMENT 8: Lake, B.G. *et al.* (2001). Lack of effect of furfural on unscheduled DNA synthesis in the *in vivo* rat and mouse hepatocyte DNA repair assays, as well as in precision-cut human liver slices. Fd Chem. Toxicol. 39: 999-1011.

ATTACHMENT 9: World Health Organization (WHO) Food Additives Series 46: FURFURAL (ADDENDUM) (2001). Summary of: Edwards (1999). "An *in vivo* Unscheduled DNA Synthesis Assay in the Mouse with Furfural". Unpublished. Report No. 3389/1/99 from BIBRA International, Carshalton (U.K.). Submitted to WHO by the Flavour and Extract Manufacturer's Association of the United States.

SUMMARIES OF SUBMISSIONS: Brief extracts of these reports follows (EPA ASSESSMENTS are found below):

ATTACHMENT I (Matsui *et al.* 1989): Furfural was among 14 aldehydes, and 20 chlorinated chemicals, assayed in the liquid *B. subtilis*/microsome rec-assay, employing the conventional strains, the recombinant-proficient H17, and the recombinant-deficient M45. Using standard procedures ±S9 metabolic action, plus the usual reference mutagens (MNNG[1], mitomycin C, ethylmethanesulfonate, 4-NQO[2], benzo(a)pyrene, 2-acetylaminofluorene, and dimethylnitrosamine). All chemicals were obtained from Wako Pure Chemical Industries, Ltd., and stated to be "JIS Special grade" (by which we assume to mean technical grades of the a.i.'s).

Furfural was reported to have no propensity to damage DNA, *i.e.*, was considered negative up to nonactivated concentrations of 1.08 x 10^3/2.45 x 10^3 *u*g/mL, and activated concentrations of 1.53 x 10^3/1.29 x 10^3 *u*g/mL (MRID 46011020, p. 18, Table 5 -DER ATTACHMENT 1).

ATTACHMENT 2 (Osawa and Namiki, 1982): Furfural was one of eight chemicals tested against a technique involving mutagen-formation resulting from the reaction with sorbic acid analogs, using the *rec*-assay with *B. subtilis* H17 and M45, or the Ames Assay with *S. typhimurium* TA98 and TA100.

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[1]MNNG, N-methyl-N[1]-nitro-N-nitrosoguanidine.

[2]4-NQO, 4-nitro-quinoline-1-oxide.

Furfural was negative up to 1.0 mg (1000 *u*g/plate) that caused 50% reduction in strains H17 and M45 (Table 1, p. 25 of MRID 46011020 - DER ATTACHMENT 2).

ATTACHMENT 3 (Phillips *et al.*, 1997): In an abstract from an oral presentation at the Cincinnati meeting of the Society of Toxicologists (SOT), the authors found furfural to be negative for the induction of UDS in hepatocytes from F344 rats orally administered the chemical up to the maximum tolerated dose (MTD), 50 mg/kg.

ATTACHMENT 4 (Hadi *et al.* 1989): Furfural was tested by the "alkaline unwinding" assay in λ-phage DNA (λ *c* 1857 S7) treated with restriction endonucleases. The authors reported that a ≥1:4 DNA base pair/furfural molar ratio caused single-strand breaks in DNA, principally in areas of AT sequences of the double-stranded DNA. However, the mechanism of DNA strand breaks remains unknown (p. 34, Tables 1 to 3 of MRID 46011020 -DER ATTACHMENT 3).

ATTACHMENT 5 (Uddin and Hadi, 1995): A repeat of the previous study (ATTACHMENT 4), employing furfural and the congener, 5-methylfurfural was performed using the same procedure. It was found that at a fixed DNA base pair/molar ratio of 1:4, furfural caused a 7-fold increase in the number of DNA single-strand breaks at 16 hours, whereas 5-methylfurfural, at the same ratio, caused approximately a 20-fold increase (Table 1, p. 42 of MRID 46011020 -DER ATTACHMENT 4). In addition, methylfurfural, but not furfural, modified DNA bases and phosphates, leading the authors to suggest that the two chemicals may cause DNA strand breaks by "different reaction mechanisms", possibly through methylation.

ATTACHMENT 7 (Kahn and Hadi, 1993): The mutagenicity of furfural was examined in the double-stranded DNA plasmid pBR322, resident in transformed competent *E. coli* HB101 cells. Plating of transformants onto ampicillin- or tetracycline-supplemented nutrient agar revealed furfural-induced mutant plasmids, as indicated by the loss of pBR322 transformation capacity at molar concentrations ranging from 5 to 20 mM Additionally, data were presented indicating that the single-strand breaks induced by furfural are repaired in the host bacterial cell. (Figure 1, p.67 of MRID 46011020 -DER ATTACHMENT 5).

ATTACHMENT 8 (Lake *et al.*, 2001): These investigators reported that they found no evidence of increased unscheduled DNA synthesis (UDS) over controls (as determined by net nuclear silver grain counts) in hepatocytes isolated from $B6C3F_1$ mice or F344 rats orally administered furfural up to their repetative maximum tolerated doses (MTDs), 320 mg/kg in mice and 50 mg/kg in rats, nor in cultured hepatocytes derived from human liver slices treated *in vitro* with 0.005 mM furfural (pp. 79 to 83 of Tables 1, 2, 4, 5, 8 and 9 of MRID 46011020 - DER ATTACHMENT 6). Cytotoxicity was seen at 10 mM furfural in the majority of donors. Although significant effects in mean net nuclear grains were seen at 2, 5, or 10 mM, these increases resulted from decreases in mean cytoplasmic grain counts due to cytotoxicity.

ATTACHMENT 9 (WHO, 2001): The WHO committee summarized A.J. Edward's (1999) BIBRA unpublished negative UDS study in mice treated up to the MTD. No original experiments were presented.

EXECUTIVE SUMMARY:

From the overall evaluation of the nine articles submitted, only three were considered **acceptable**. Five of the remaining studies were **unacceptable** for various reasons (*i.e.*, lack of purity information, presented in abstract form, no primary data, or only a summary of a UDS assay). The FEMA summary (Adams *et al.*, 1997) has already been reviewed and assessed by the Agency (MRID 46011019), and found to be **unacceptable.**

Findings from the acceptable studies (Matsui *et al.*, 1989; Osawa and Namiki, 1982; Lake *et al.*, 2001) show that furfural did not induce DNA damage/repair in the *B. subtilis* assay, was not mutagenic in *S. typhimurium* TA98 and TA100, and did not induce UDS in mice and rats or in cultured human hepatocytes. These findings are supported by the lack of a positive response in several of the unacceptable assays, which included UDS, alkaline unwinding, or assays dealing with modifications of DNA bases and phosphates. Although there was evidence of lesions in the double-stranded DNA of the plasmid pBR322, repair of this damage takes place when the plasmid was propagated in the host cell. Two of the unacceptable studies (Hadi *et al.*, 1989; Uddin and Hadi, 1951) showed that furfural induced single strand breaks in calf thymus DNA.

EPA ASSESSMENTS/CLASSIFICATIONS OF INDIVIDUAL STUDIES:

Following are assessments and classifications of the individual studies:

Matsui *et al.*, 1989 (ATTACHMENT 1): Although some minor criteria for acceptance are missing from this publication, we accept the investigators' conclusion that furfural (among other chemicals) is negative in the *B. subtilis* rec-assay, on the basis of adequate procedures and purity/source of the chemicals provided. Thus, the study is classified: **Acceptable/Guideline** for bacterial (DNA damage) data.

Osawa and Namiki, 1982 (ATTACHMENT 2): The procedures employed appear adequate for the intended investigation. However, the statement that "all chemicals used in these experiments were of guaranteed grade" is insufficient as a guarantee that they were technical grade. However, it was further offered that furfural and sorbic acid methyl ester were "purified by vacuum distillation," which produced the technical grade chemicals. Therefore,, we accept the negative results, and classify this assay for furfural **acceptable/**but **non-guideline**, since studies of this type are not included in the FIFRA Test Guidelines.

Phillips *et al.*, 1987 (ATTACHMENT 3): This abstract reporting negative results for UDS in hepatocytes drawn from rats (source and sex, not provided) administered furfural "up to an MTD level" is not fully supportable by the submission, *i.e.*, **unacceptable.**

Hadi *et al.* (1989) ATTACHMENT 4): The novel procedures for determining strand breakage induced in lambda phage duplex DNA by furfural (in the main by reacting with AT sequences) is well presented, but the lack of providing purity or description of the chemical renders the overall conclusions of this type of study **UNACCEPTABLE.**

Uddin and Hadi, 1995 (ATTACHMENT 5): The further exploration of the novel methodology mentioned in ATTACHMENT 4 is also **UNACCEPTABLE** for the same omissions as mentioned above.

Adams *et al.* 1997 (ATTACHMENT 6): [Already reviewed and assessed in MRID 46011019, ATTACHMENT 1].

Kahn and Hadi, 1993 (ATTACHMENT 7): Examination of the action of furfural inducing single-strand breaks in the plasmid pBR322 revealed several mutant plasmids. The study, however, is **UNACCEPTABLE** for the same omissions as mentioned above.

Lake *et al.*, 2001 (ATTACHMENT 8): The meticulous procedures reported in this publication support the authors' conclusions that furfural is negative in inducing UDS in hepatocytes isolated from mice and rats treated up to their MTDs, as well as the lack of genotoxicity *in vitro* in human livers. Since the source *(International Flavors and Fragrances,* , Union Beach, NJ) and purity (≥98%) of the chemical is provided, all major criteria for the requirements of the FIFRA Test Guidelines are satisfied; and thus the publication is classified **acceptable/guideline**.

WHO, 2001 (ATTACHMENT 9): **Unacceptable**, since it is only a review of another investigator's unpublished study.